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Évaluation du devenir et des impacts des microplastiques dans l'environnement terrestre avec l'escargot *Cantareus aspersus*

Romain Colpaert

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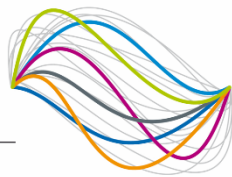
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Par

M. Colpaert Romain

Evaluation du devenir et des impacts des microplastiques dans l'environnement terrestre
avec l'escargot *Cantareus aspersus*

Thèse présentée et soutenue à Besançon, le 10 septembre 2024

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« Il est dur d'échouer ; mais il est pire de n'avoir jamais tenté de réussir »

Franklin D. Roosevelt.

Avant-propos

La présente thèse vise à acquérir des connaissances pour l'évaluation du devenir des microplastiques (MPs) et de leurs impacts sur les écosystèmes terrestres avec le bioindicateur de la qualité des sols *Cantareus aspersus*. Cette thèse combine à la fois un volet visant à acquérir des connaissances pour l'évaluation des effets des MPs, seuls ou en co-exposition avec des éléments traces métalliques (ETMs), chez *Cantareus aspersus*, et un volet utilisant une méthode de quantification indirecte des MPs pour évaluer leur transfert le long des réseaux trophiques. La thèse est organisée en trois axes scientifiques complémentaires :

1. L'acquisition de connaissances sur les potentiels écotoxiques de différents MPs sur *Cantareus aspersus*, soit en fonction de la taille des particules, soit en fonction de la nature polymérique des MPs, lors d'essais d'écotoxicité sub-chronique ou à l'échelle du cycle de vie.
2. La caractérisation de la capacité des MPs à moduler la disponibilité environnementale de différents éléments traces métalliques (ETMs) dans les sols, ainsi que leur bioaccumulation dans les tissus de *Cantareus aspersus*.
3. L'évaluation de la capacité des MPs à être transférés le long de réseaux trophiques, avec le positionnement de *Cantareus aspersus* soit en tant que consommateur apical, soit en tant que niveau intermédiaire dans le réseau trophique.

La structure du manuscrit de thèse est la suivante : une introduction générale concernant les MPS suivie par des chapitres et sous chapitres correspondant à des articles de recherches acceptés, soumis ou en cours de préparation (en anglais avec le résumé en français). Chacun de ces chapitres comporte une introduction qui complète les thèmes abordés en introduction générale, une section spécifique de matériels et méthodes, une partie résultats, une discussion et une conclusion. Enfin la dernière partie du manuscrit propose une discussion et une conclusion générale de l'ensemble des chapitres de la thèse. Une bibliographie spécifique à l'introduction générale, aux différents chapitres et la discussion générale terminera chacun des chapitres de ce manuscrit afin de faciliter la lecture et la recherche de l'ensemble des références, et seront associées à une bibliographie générale en fin de thèse.

Liste des publications et participations aux congrès

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Colpaert R., de Vaufleury A., Crini N., Amiot C., Gimbert F., **Influence du polystyrène sur la disponibilité environnementale de l'As, du Cd et du Hg dans les sols.** COLLOQUE DE LA SOCIETE FRANCAISE D'ECOTOXICOLOGIE FONDAMENTALE ET APPLIQUEE 2022, Metz (FR), Communication sous forme de poster.

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Colpaert R., de Vaufleury A., Rieffel D., Amiot C., Crini N., Gimbert F., **Les effets des microparticules de polystyrène sur la disponibilité environnementale et la biodisponibilité de l'arsenic, du cadmium et du mercure depuis les sols jusqu'à l'escargot *Cantareus aspersus*.** COLLOQUE DE LA SOCIETE FRANCAISE D'ECOTOXICOLOGIE FONDAMENTALE ET APPLIQUEE 2024, Besançon (FR), Communication orale.

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Partie A – Introduction générale

I. Historique

Les plastiques peuvent être succinctement définis comme étant des polymères synthétiques ou semi-synthétiques, fabriqués par l'être humain. Le terme de polymère provient des termes grec πολύς (polus), signifiant « plusieurs » et μέρος (meros), signifiant « partie » ; un polymère peut être défini comme étant une macromolécule de haute masse moléculaire, formée de répétitions de molécules de plus faible masse moléculaire, appelées monomères. Ces monomères étant les « unités constitutionnelles » des macromolécules, et les formant par un processus appelé « polymérisation » (Jones et al., 2009). Bien que l'usage de substances polymériques par l'être humain soit antérieur à celui des plastiques, avec l'utilisation de polymères naturels tels que les fibres de cellulose (bois et papier, fibres naturelles), les résines ou les caoutchoucs naturels (comme les latex), la date d'apparition des premiers polymères considérés comme étant des plastiques est encore sujette à débat. Le début de « l'ère des plastiques » remonte soit à 1869, avec l'invention par John Wesley et Isaiah Hyatt du celluloïd (Newton, 2021), soit à l'apparition de l'ébonite (issu d'un processus de vulcanisation du latex avec 40 à 60 % de soufre adjoint) comme premier thermoplastique, avec les brevets déposés par Thomas Hancock en Angleterre en 1843 et par Charles Goodyear aux Etats-Unis en 1844 (Gilbert, 2017). D'autres sources placent encore le début de « l'ère du plastique », avec la synthèse du premier thermoset (défini plus tard au cours de ce chapitre) par Leo Baekeland via la polycondensation de phénols avec du formaldéhyde, donnant la bakélite en 1907. De ces découvertes ont suivi le développement de nouveaux polymères jusqu'à la seconde guerre mondiale, tels que le polychlorure de vinyle (PVC) via la photo-polymérisation de monomères chlorure de vinyle (brevet déposé par Friedrich Heinrich August Klatte en 1913), la synthèse des premiers polyesters saturés en 1929 (ayant donné par la suite la création polyéthylène téréphtalate en 1955), les premières productions du polyméthyl méthacrylate (PMMA) en 1930 par l'Imperial Chemical Institute au Royaume-Uni et en 1933 par Otto Röhm, la création des premières résines époxy en 1934 et du polyamide 6 (ou nylon 6) en 1938 par Paul Schlack, après la création du nylon 6-6 en 1935 par Wallace Carothers pour la compagnie américaine DuPont de Nemours (Gilbert, 2017; Hermes, 1996), ou la création du polystyrène par Herman Staudinger dans les années 1930, ou encore le développement des fluoro-polymères avec la synthèse en 1938 du polytétrafluoroéthylène, ou celle du polyéthylène en 1939, par exemple (Feldman, 2008). Cependant, malgré ces avancées, la production de plastique n'était jusque-

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là que marginale, l'envolée de leur utilisation n'ayant eu lieu qu'à partir de la seconde guerre mondiale, avec l'intervention des Etats-Unis. Cette guerre a eu pour effet de compromettre les approvisionnements en caoutchouc des Etats-Unis, qui ont vu 90 % de leurs accès à cette matière compromis. Dès lors, le président américain Franklin D. Roosevelt créa le « Rubber Reserve Company (RRC) Program » en Juin 1940 puis le « Rubber Survey Committee » en Août 1942, qui furent des comités industriels et scientifiques en charge de régler les problèmes d'approvisionnement en polymères naturels, via l'établissement d'une industrie de production de polymères synthétiques. Ces comités établirent une production de masse de caoutchoucs synthétiques via l'industrialisation de la production de styrène-butadiène (SBR), de PMMA et de polyéthylène (découvert en 1939), afin de combler à la fois les besoins militaires (vitrages aéronautiques, pneumatiques, matériel léger et résistant), et les besoins civils. Conséquemment, la production américaine est passée de 2 241 à 920 000 tonnes de caoutchouc synthétiques entre 1942 et 1945 (American Chemical Society, 1998; Gilbert, 2017; Tickner et al., 2021). A la fin de la guerre, l'industrie plastique américaine comptait quelques 370 compagnies, dont quelques grands noms encore connus aujourd'hui, tels que : the Dow Chemical Company, General Electric, Du Pont, Bakelite, Monsanto, Phillips Petroleum, Eastman Kodak ou Boonton Molding Company. Fortes de leur expérience acquise durant la guerre, ces compagnies se tournèrent dorénavant vers le marché du consommateur américain (Johnston, 2018). Les suites de la seconde guerre mondiale ont donc vu l'expansion des produits à base de plastique, de par la création continue de nouveaux polymères, ou la baisse du coût de production de polymères préexistants, ces produits offrant soit des substituts bon marché aux matériaux traditionnels, soit des matériaux présentant des propriétés intéressantes, dans une économie de reconstruction après la guerre, puis dans une société de consommation. En effet, les plastiques offraient de toutes nouvelles perspectives, tant en termes d'innovation technologique (durabilité et résistance des matériaux, possibilité d'obtenir tout type de forme ou de couleur, aspect hygiénique des plastiques) qu'en terme de baisse des coûts de production, et donc de baisse des prix pour les consommateurs, laissant refléter une société basée sur une consommation sans limite de produits faits à sa convenance, menant à la « transformation des modes de vie » (Johnston, 2018; Meikle, 1997; Thompson et al., 2009b, 2009a). Tel que dit par Susan Freinkel : « Produit après produit, marché après marché, les plastiques ont remis en question les matériaux traditionnels et ont

gagné, prenant la place de l'acier dans les voitures, du papier et du verre dans les emballages, et du bois dans le mobilier » (Freinkel, 2011).

II. Les microplastiques

II.1. Définition des plastiques

Pour une définition plus précise, les matériaux plastiques sont donc des polymères semi-synthétiques ou synthétiques, correspondant à la structure chimique de base du matériau, de diverses natures, et présentant des propriétés physicochimiques variées. La caractéristique principale de ces polymères plastiques est qu'ils sont malléables à haute température – ils ont une plasticité – et peuvent donc être moulés pour obtenir des objets solides. A ces polymères plastiques sont également associées des substances chimiques qualifiées d'additifs, servant à modifier les propriétés physico-chimiques des polymères de base, afin de leur conférer les propriétés désirées pour les usages auxquels ils sont destinés. En dépit de ces différentes compositions, tous les plastiques ont une structure de base hydrocarbonée, qu'elle soit dérivée de produits pétroliers (pétrole ou gaz) ou issue de sources biologiques, par exemple de l'amidon, des huiles végétales ou des sucres (Ali et al., 2023; European Environment Agency, 2021).

Ces plastiques peuvent être classés selon trois grandes catégories :

- Les thermoplastiques, dans lesquels les chaînes polymériques sont non réticulées, et sont associées par des forces d'attraction intercaténaire, ce qui leur permet de se déplacer indépendamment les unes des autres, et qui faiblissent rapidement avec l'augmentation de la température, donnant un état visqueux réversible avec l'abaissement de la température (ils conservent la réversibilité de leur thermoplasticité initiale). Au-dessus de leur température de transition vitreuse et en dessous de leur température de fusion, les propriétés des thermoplastiques changent drastiquement sans changement de phase. Les thermoplastiques peuvent être catégorisés en thermoplastiques amorphes ou semi-cristallins, en fonction de l'arrangement de leurs chaînes moléculaires. Les thermoplastiques amorphes possèdent une structure non organisée quand les thermoplastiques semi-cristallins possèdent une structure partiellement organisée, avec certaines régions où les chaînes moléculaires s'alignent les unes

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par rapport aux autres. Les thermoplastiques amorphes sont plus cassants, et moins résistants au stress chimique et environnemental que les thermoplastiques cristallins. Cependant, tous les thermoplastiques ont un comportement flexible, et peuvent être chauffés à nouveau pour leur donner une nouvelle structure et une nouvelle forme, leur permettant d'être recyclés (Eyerer, 2010; PAPREC, 2023; Schweizer, 2017).

- Les thermodurcissables, qui sont des polymères hautement réticulés, dans lesquels les chaînes de polymères sont liées entre elles soit chimiquement (via l'action de la chaleur, soit par un agent durcissant), soit physiquement (ex : par irradiation d'un prépolymère, substance visqueuse contenant des groupes réactifs qui donneront le polymère final après catalyse), et forment un réseau en trois dimensions, empêchant un mouvement indépendant des chaînes polymériques (présenté en Figure 1). Cette réticulation permet une meilleure résistance mécanique, thermique et chimique que pour les thermoplastiques. Cependant les thermodurcissables ne permettent pas après fabrication d'être refondus (ils perdent leur thermoplasticité initiale de par la présence de nœuds covalents intercaténaire), et ne pourront donc pas voir leur structure modifiée, empêchant généralement leur recyclage (AAT Bioquest, 2022; Eyerer, 2010; Horie et al., 2004; MARMON Aerospace & Defense, 2022; PAPREC, 2023).

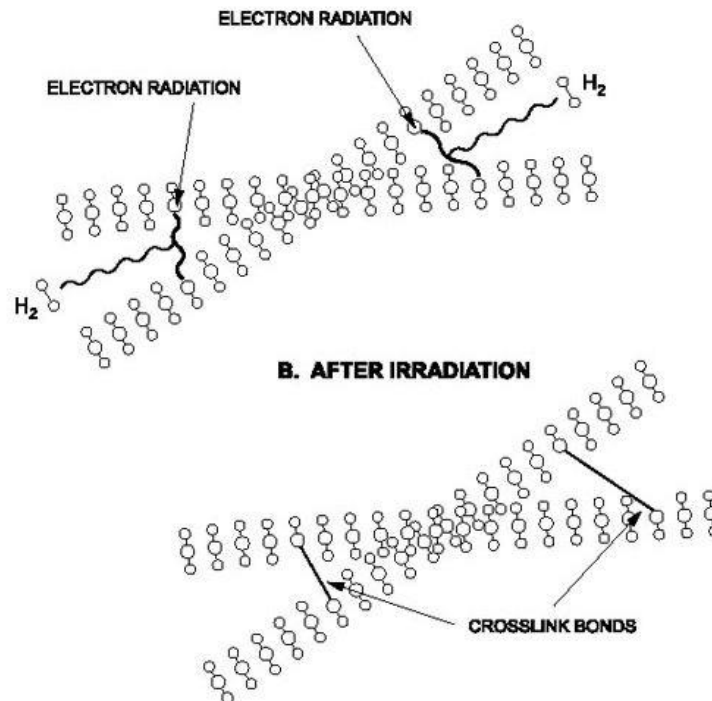


Figure 1: Formation de liens intercaténaire lors de la phase de réticulation des plastiques thermodurcissables (ici une réticulation par irradiation). L'excitation des monomères, ici via un rayonnement, permet de créer des ponts covalents entre les chaînes polymériques (« crosslinking » en anglais) donnant la structure rigide des plastiques thermodurcissables. MARMON Aerospace & Defense.

- Les élastomères sont des polymères thermodurcissables possédant de grandes chaînes polymériques faiblement réticulées, avec de faibles forces de cohésion entre les chaînes moléculaires, leur donnant un comportement viscoélastique au-dessus de leur température de transition vitreuse. Cette faible réticulation leur permet d'avoir une structure amorphe en l'absence de contrainte mécanique tout en permettant un dépliement et une structuration réversible (cristallisation) des chaînes polymériques pendant une contrainte de traction allant dans le sens de ces chaînes ; avec un retour à un état amorphe sous l'effet des mouvements browniens dans les chaînes moléculaires en l'absence de contrainte de traction, leur donnant un comportement viscoélastique (BUFA, 2024; Ciullo and Hewitt, 1999; Gent, n.d.; Kutz, 2002; Polymer Science Learning Center, n.d.). Les différences entre thermoplastiques, thermodurcissables et élastomères en termes de réticulation sont présentées en Figure 2.

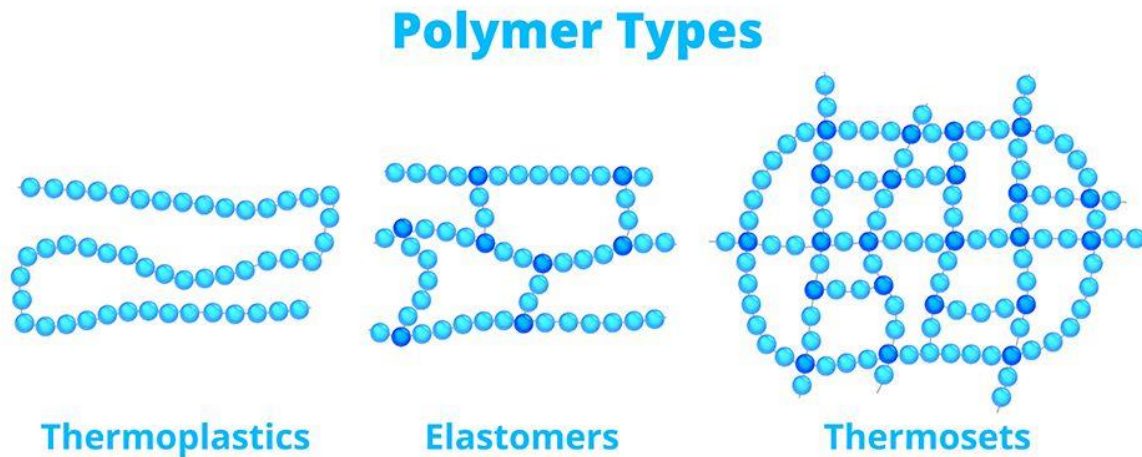


Figure 2: Représentation schématique des niveaux de réticulation des thermoplastiques (« Thermoplastics »), des élastomères (« Elastomers ») et des thermodurcissables (« Thermosets »).
Crédit : BUFA Thermoplastic Composites.

Remarque : Le recyclage des polymères plastiques se fait traditionnellement via des techniques de refonte à haute température, empêchant le recyclage des thermodurcissables à cause de scissions dans leur chaîne polymérique. Cependant, de nouveaux processus de recyclage sont en cours de développement, via l'utilisation de techniques de dépolymérisation pour obtenir des monomères pouvant être repolymérisés (Fortman et al., 2018).

Ces grandes familles de plastiques sont elles-mêmes subdivisées en différentes catégories, selon leur structure chimique de base. Pour dresser une vue d'ensemble, les principales classes de polymères en termes de production et de consommation font parties de la famille des thermoplastiques, ces principales classes étant les polyoléfines, les polystyréniques, les polyamides, les polyvinyliques et les polyesters linéaires (Plastics Europe, 2022). Les polysulfones et les polycarbonates, bien que moins produits, sont importants en tant que polymères techniques :

- Les polyoléfines (ou polyalcènes), comprenant le polyéthylène (PE) et le polypropylène (PP), et qui sont produites par polymérisation de l'éthylène et du propylène, respectivement. Cette famille est largement utilisée, et représente à elle seule environ 46 % de la masse de plastique produite (Figure 3). Le PE peut, selon son degré de ramification, et donc sa densité, être catégorisé en PE basse densité (LDPE, « Low-Density Polyethylene »), en PE haute densité (HDPE, « High-Density Polyethylene ») ou en PE linéaire à faible densité (LLDPE, « Linear Low-

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Density Polyethylene »), le degré de ramification allant croissant entre le LLDPE, le HDPE et le LDPE. Les polyoléfinés sont par leur nature chimique hydrophobes, possèdent une grande inertie chimique, et sont compatibles avec l'utilisation au contact de produits alimentaires (Guillemot and Patrascu, 2019; Plastics Europe, 2024a).

- Les polystyréniques, qui comprennent principalement le polystyrène (PS), ainsi que ses dérivés, qui sont produits par polymérisation du styrène. De formule générale $\text{CH}_2\text{-CH(Ph)}_n$, pour le PS, ces composés sont relativement cassants, avec une faible résistance à la contrainte mécanique et une faible résistance aux attaques chimiques, de par la présence de composés phénoliques. Cette famille est l'une des plus utilisées (Maafa, 2021), avec une production totale entre 24 et 28,5 millions de tonnes depuis les années 1950, et contamine donc largement les environnements. Le principal représentant de cette classe est le polystyrène, qui peut se présenter sous forme dite « cristal » transparent, sous forme « choc » opaque mais avec une meilleure résistance aux chocs (via ajout de butadiène dans le milieu réactif pendant la polymérisation), ou sous forme dite « expansée » via ajout d'un agent d'expansion pendant la polymérisation. Ces différents procédés produisent du PS utilisable en matériau d'emballage (imperméabilité, résistance aux chocs), en tant qu'isolant thermique, ou encore pour la fabrication de consommables à usage unique comme la vaisselle jetable (Société Chimique de France, 2024).

- Les polyamides (PA), polymères dont la formule chimique contient des fonctions amides de façon régulière. Ces plastiques représentent 7 % de la production mondiale de fibres synthétiques, 30 % des plastiques techniques, et sont très utilisés dans l'industrie automobile, électrique et électronique, pétrochimique, agro-alimentaire ou textile (Goncalves, 2011; Wesołowski and Płachta, 2016). Ils sont obtenus généralement par polycondensation d'un acide dicarboxylique avec une diamine, leur nomenclature se présente sous la forme de nombres XY indiquant le nombre d'atomes de carbone dans la diamine utilisée (X) et dans l'acide utilisé (Y) pour la synthèse du polymère.

- Les polyvinyliques, qui sont des polymères de formule $\text{CH}_2=\text{CHX}$, où X représente un groupe comme le chlore, l'acétyle, l'acide ester etc. Cette classe de polymère comprend le polychlorure de vinyle (PVC) ainsi que des dérivés tels que les copolymères de chlorure de vinyle et d'acétate de vinyle (polyacétochlorure de vinyle, PEVA), le polychlorure de vinylidène (PVDC), le polyacétate de vinyle (PVAC), ainsi que le polybutyral et le polyformal

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de vinyle (PVB et PVFM). Ces composés sont utilisés en revêtement, en isolants, ou en contenants de toute sorte (Centre National de Ressources Textuelles et Lexicales (CNRTL); Plastics Europe, 2024b; Polymer Science Learning Center).

- Les polycarbonates, qui sont issus d'une polymérisation par condensation entre le bisphénol A et soit le carbonate de diphenyle soit l'oxychlorure de carbonate (ou phosgène). Les polycarbonates sont résistants aux chocs et à la chaleur, ininflammables et transparents, et leurs applications sont variées, allant du matériel médical aux applications électroniques et électriques, ou encore la construction ou l'équipement automobile (The Essential Chemical Industry, 2017).

- Les polysulfones (PSFs) et polyéther sulfones (PESs), issus de la polymérisation par substitution nucléophile d'un diphenol (classiquement le bisphénol A) et du bis(4-chlorophényl sulfone) sont des polymères qui contiennent des groupes éther et sulfone (PESs) ou des groupes éther, sulfones et alkyl (PSFs). Ces polymères possèdent une grande résistance mécanique, thermique et chimique (Igder et al., 2020; Raja Rafidah et al., 2020) et sont utilisés en tant que polymères techniques pour des applications de pointe comme la recherche scientifique ou les applications médicales.

- Les polyesters linéaires sont des polymères obtenus par polycondensation par estérification de acides dicarboxyliques avec un diol. Cette classe comprend pour les polymères les plus utilisés le polyéthylène téréphtalate (PET), ainsi que l'acide polylactique (PLA), ce dernier ayant été extensivement étudié et utilisé, étant un polymère dit biodégradable et biosourcé. La plupart des polyesters linéaires sont des polymères aromatiques-aliphatiques. Leurs propriétés (haute hydrophobie, résistance au stress chimique, mécanique et thermique, faible conductivité électrique, grande stabilité) permettent une utilisation pour l'emballage agro-alimentaire, l'industrie textile, électrique ou automobile, par exemple (British Plastics Federation, 2024; Hubner, 2021).

II.2. Marqueurs socio-économiques

Depuis le début des années 1950, la production mondiale annuelle de plastique est passée d'environ deux millions à 391 (estimation basse) ou 460 millions de tonnes (estimation haute),

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les estimations basses ne prenant pas en compte les quantités utilisées dans la fabrication de fibres textiles, adhésifs, et produits d'application de surface (Letcher, 2020; OECD, 2022; Plastics Europe, 2022). Cette production est portée à plus de 50 % par l'Asie (la Chine comptant pour près du tiers de la production mondiale), 18 % par l'Amérique du Nord et 15 % par l'Europe occidentale, 8 % par l'Afrique et le Moyen-Orient, 4 % par l'Amérique du Sud et 3 % par l'Europe orientale. Comme décrit en Figure 3, environ 46 % de cette production mondiale correspond à des polyoléfines, 13 % à des polyvinyliques, 6% à des polyesters linéaires et 5 % à des polystyréniques ; tous issus de dérivés pétrolifères non recyclés. Les produits recyclés correspondant à environ 8 % de la production mondiale, et les bioplastiques à 1,5 % de cette production. Les principales applications de ces plastiques concernent l'emballage (44 %), la construction (18 %), l'industrie automobile (8 %), l'industrie électrique et électronique, l'ameublement, les loisirs et le sport (7 %), et la production alimentaire (4 %), le reste de la production étant utilisée pour des applications tierces.

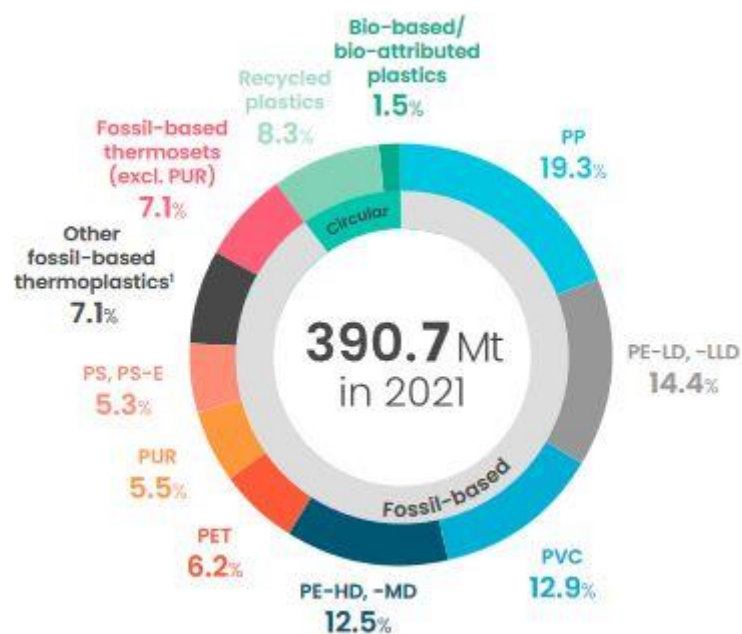


Figure 3: Pourcentages de la production mondiale des principaux types de plastiques. Les polyamides sont compris dans les « Other fossil-based thermoplastics » (volume de production inférieur à 5 % de la production totale). PUR = Polyuréthane. Plastic – The Facts, 2022

Cette augmentation de la production passée et projetée (les estimations prévoyant un triplement des quantités de plastiques utilisés, avec 1230 millions de tonnes de plastiques utilisées à l'horizon 2060) provient majoritairement de deux facteurs : 1) l'augmentation de la population mondiale (avec une population passant de 2,5 milliards de personnes en 1950 à

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une population projetée à 10 milliards aux alentours de l'an 2050 (United Nations, 2022), et II) l'augmentation progressive du produit intérieur brut dans tous les pays, passant d'un PIB *per capita* de 455,6 dollar (USD) en 1960 à 12743 USD en 2020 et avec une augmentation de la croissance projetée de cet indicateur, associée à une augmentation des niveaux de vie, et donc de la consommation de produits manufacturés (OECD, 2022; World Bank).

L'industrie plastique est donc aujourd'hui toujours en expansion. Cette industrie représente environ un million et demi d'emploi sur le territoire européen, et autant sur le territoire américain, se classant comme la huitième plus grande industrie en Europe et la sixième aux Etats-Unis, les statistiques concernant les autres régions n'étant pas accessibles (Plastics Europe, 2022; Plastics Industry Association, n.d.; U.S. Bureau of Labor Statistics, 2022).

Cette tendance est due à l'utilité sociétale des plastiques, ceux-ci offrant des avantages à tous les stades de leur cycle de vie. En effet, leur utilisation est avantageuse, leurs propriétés physicochimiques étant idéales pour de multiples applications indispensables à nos sociétés, comme par exemple les technologies nécessitant des polymères légers et résistants (transport, aéronautique, recherche spatiale) ou pouvant être stérilisés (utilisation dans le matériel de recherche et matériel médical). De plus, leur production, comme vu précédemment, est un grand marché économique et professionnel, leur collecte et leur recyclage étant également porteuses d'emploi et générant une croissance économique, notamment pour des pays à indice de développement humain moyens à faibles. Cependant les contreparties sont nombreuses, notamment une détérioration de la santé des habitants proches des centres pétrochimiques pour la production, un risque sur la santé des consommateurs, notamment via les additifs utilisés dans les produits plastiques à usage humain (exemple du Bisphénol A dans les produits destinés à l'utilisation alimentaire), le coût économique lié au recyclage pour les communautés, et le risque de contamination des environnements terrestres et aquatiques par les plastiques et leurs produits dérivés, à savoir les microplastiques, nanoplastiques et les xénobiotiques associés (Karasik et al., 2023).

Les plastiques sont produits en grande quantité et sont maintenant utilisés dans de nombreuses activités humaines. Cependant le risque de contamination des environnements par ces plastiques est grand. Bien que le devenir de certaines familles de

plastiques (polyoléfines, polystyréniques) soit relativement documenté, leur volume de production les rend accessibles à tous les environnements, et des efforts de recherches sur leurs potentiels impacts sont nécessaires. D'autres familles, comme les polyamides, bien qu'ayant une production plus faible peuvent aussi largement contaminer l'environnement. Cependant les efforts de recherche envers ces familles restent limités et l'exploration de leurs effets nécessiterait des études supplémentaires.

III. Des plastiques aux microplastiques

La fin de vie des plastiques est problématique, et il est estimé que seuls environ 21 % des plastiques utilisés sont recyclés ou incinérés au niveau global, le reste étant soit mis en décharge soit rejeté dans l'environnement (Helm et al., 2023; UNEP, 2021). Une fois que ces plastiques ont atteint l'environnement, ils subissent un vieillissement, suite à plusieurs facteurs, qui peuvent être abiotiques ou biotiques. Dans les facteurs abiotiques, l'irradiation UV est le principal processus de vieillissement des plastiques, les stress thermiques et chimiques en étant les autres grandes causes. Ces stress vont permettre une dégradation des plastiques, principalement via photo-oxydation ou oxydation, ce qui va induire une augmentation de la cristallinité des plastiques, réduire leur poids moléculaire, et induire la synthèse de composés réactifs à l'oxygène, produire une déshydrochlorination de certains polyvinyliques comme le PVC, voire produire des scissions de chaînes au sein des polymères. Ces polymères photo-oxydés vont ensuite subir des phénomènes de fragmentation ou d'abrasion de surface, à cause de stress chimiques, et surtout mécaniques, libérant des particules de petite taille, appelés microplastiques (Andrady, 2017; Andrady et al., 2022; Zhang et al., 2021).

Le terme de microplastique (MP) a d'abord été introduit par Thompson et al. (2004), pour décrire l'accumulation de petites particules de plastiques dans les milieux marins. Suite à cela, une taille maximale pour les MPs fixée à 5 mm été proposée par Arthur et al. en 2009. Depuis, de nombreuses propositions ont été faites, pour définir les tailles associées aux macroplastiques (MaP), mésoplastiques (MeP), MPs et nanoplastiques (NPs). A l'heure actuelle, aucun consensus n'a été encore trouvé dans la communauté scientifique concernant la limite inférieure du diamètre des microplastiques, la NOAA classant tous les plastiques sous

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5 mm comme étant des microplastiques, quand le GESAMP les catégorise comme étant compris entre 5 mm et 1 μm , alors que l'EFSA retient une taille entre 5 mm et 0.1 μm (Frias and Nash, 2019; Hartmann et al., 2019).

La définition en terme de taille retenue pour les microplastiques dans cette thèse est donc celle de l'agence européenne, l'EFSA, pour des tailles de particules comprises entre 5 mm et 100 nm et pour les nanoparticules, une taille limite de 100 nm, tout comme la définition ISO (European Commission, 2011; European Commission. Joint Research Centre. Institute for Health and Consumer Protection et al., 2010; International Standardization Organization, 2015).

Ces MPs peuvent être dits soit « primaires », s'ils ont été produits et commercialisés dans cette gamme de taille ; ce qui est le cas des MPs pour des applications dans des produits pharmaceutiques et cosmétiques, fabriqués à des fins de recherche, ou pour des utilisations pour du sablage à air comprimé, par exemple ; soit dits « secondaires » lorsqu'ils proviennent de la fragmentation de plastiques de plus grande taille (Cole et al., 2011).

Ces MPs peuvent également être catégorisés selon leur forme. Ces particules pouvant être des fragments, des granules, des films, des filaments, des mousses, des microbilles, des sphères, etc. Cependant, aucune normalisation n'a été effectuée, bien que le GESAMP ait recommandé de classer ces particules en cinq grandes catégories, qui sont :

- Les fragments, particules solides et irrégulières, semblant provenir de la fragmentation de morceaux plus gros, comprenant les granules et les paillettes,
- Les mousses, particules quasi-sphériques ou granulaires, qui peuvent se déformer sous la pression et peuvent être élastiques, dépendamment de leur état de vieillissement,
- Les films, particules plates et flexibles, avec des bords lisses ou anguleux,
- les MPs ligneux, qui sont de longues fibres dont la longueur est substantiellement plus grande que leur largeur, comprenant les fibres, les filaments et les brins,
- et les pastilles ou billes, qui sont des particules solides, sphériques, avec une surface lisse ou granuleuse.

Cette forme des MPs peut donner une indication de leur origine, ainsi une microbille peut indiquer un MP primaire utilisé dans des produits cosmétiques, alors qu'une fibre pourra indiquer une origine plutôt textile (GESAMP, 2019; Rosal, 2021). De la forme de ces particules pourra également être déduit un comportement potentiel, notamment en milieu aquatique ; les particules de type fragments ou billes étant susceptibles de sédimenter plus rapidement que les films et les fibres, de par leur différence dans leur ratio masse sur volume.

De par leur origine et leurs caractéristiques, les MPs peuvent s'accumuler dans tous types de milieu, incluant les eaux, les sols, et l'air, que ce soit en zones urbaines fortement anthropisées, ou dans des milieux ruraux ou naturels faiblement voire non anthropisés, et ainsi exercer leurs effets sur l'environnement et le biote. Comme soulevé par He et Luo, (2020), un certain nombre de questions concernant l'occurrence et les effets des MPs, notamment dans les environnements terrestres restent à être élucidées. Aussi le travail apporté durant cette thèse se concentrera sur les effets des MPs, à l'exclusion des NPs, dans les environnements terrestres.

III.1. Le cycle des microplastiques

La grande majorité des plastiques sont fabriqués, utilisés, et rejetés en milieu terrestre à l'exception des activités de pêche (Horton and Dixon, 2018). On estime que les milieux terrestres sont 4 à 23 plus contaminés que les milieux aquatiques (Horton et al., 2017). Les milieux terrestres sont contaminés, de par soit des pertes durant la collecte et le retraitement des plastiques (Basuhi et al., 2021), des pertes industrielles de plastique ou de MPs primaires pendant la production, le transport ou l'utilisation de produits contenant des plastiques (Karlsson et al., 2018; Kurniawan et al., 2021; Lechner and Ramler, 2015), une fuite de matière depuis les décharges et sites d'enfouissement (Yadav et al., 2020), de par des activités, agricoles, notamment via l'utilisation de paillage plastique (Bai et al., 2024; Ren et al., 2021; L. Xu et al., 2022; Z. Zhang et al., 2022a), l'abrasion des pneumatiques et des revêtements routiers (particules déposées, peintures, etc...) durant la circulation automobile (Cunningham et al., 2022; Giechaskiel et al., 2024; Jannah et al., 2024; Kabir et al., 2024; Müller et al., 2022; Tamis et al., 2021) entre autres. D'autres sources importantes de MPs en milieu terrestre sont les produits de soin personnels, comme les exfoliants et les dentifrices, l'Asie étant le principal

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contributeur à cette source de MPs (Protyusha et al., 2024), ainsi que des particules provenant de nos biens intérieurs (revêtements mobiliers, peintures, serviettes, tapis, etc...) ou de nos vêtements, ceux-ci contenant souvent des fibres plastiques, ces éléments participant à la contamination de l'air intérieur (Dris et al., 2017; Fang et al., 2024; Shao et al., 2022). Les textiles eux-mêmes sont problématiques, car ils relarguent des microfibres plastiques dans les milieux terrestres durant leur fabrication, leur utilisation et leur fin de vie, mais également les milieux dulcicoles durant leur lavage (Hernandez et al., 2017; Świątek and Dąbrowska, 2024; Yang et al., 2019; Y.-Q. Zhang et al., 2022).

Les milieux aquatiques, dulcicoles comme marins sont eux aussi exposés aux contaminations par les MPs, soit via une contamination aéroportée de plastiques et MPs depuis le milieu terrestre (49 à 2500 particules/L), soit via le ruissellement des eaux de surfaces se chargeant en particules plastiques (de 0,38 à 197.000 particules/L), le lavage des items textiles (vêtements, etc...), soit directement via une contamination directe par les eaux usées (Dris et al., 2015; Shafi et al., 2024; Y.-Q. Zhang et al., 2022). Ce dernier point est d'importance, en effet bien que les eaux usées soient traditionnellement retraitées dans des stations d'épurations, celles-ci restent problématiques. En effet, les estimations donnent entre 15 et 51 trillions de particules flottant dans les océans (Uddin et al., 2021). Ces particules provenant essentiellement de la décharge fluviale d'effluents, traités ($1,47 \cdot 10^{15}$ particule/an) comme non traités ($38,5 \cdot 10^{15}$ particules/an), contribuant à une valeur estimée de 78 % de la contamination océanique. Le reste de la contamination des milieux aquatiques étant attribué à des sources plus diffuses provenant du milieu terrestre comme la déposition aéroportée et le ruissellement de plastiques (Wang et al., 2024).

Une fois dans les eaux usées, les MPs se retrouvent dans les stations d'épuration (dans les territoires possédant de telles structures), et bien que ces stations puissent retenir jusqu'à 95 % des particules dans l'eau, une partie est rejetée dans les cours d'eau, les contaminant ainsi, jusqu'à l'océan (Komorowska-Kaufman and Marciniak, 2024; Li et al., 2018). Cette contamination dépend de l'efficacité des stations d'épuration, mais le nombre de particules déchargées peut varier de $5 \cdot 10^5$ à $1,4 \cdot 10^{10}$ particules par jour et par station d'épuration, avec des rejets estimés selon les études à $1,60 \cdot 10^8$ particules.jour⁻¹ pour l'une des plus grosses stations d'Italie (Ahmed et al., 2024), entre 0,28 et 0,47 particules.m⁻³ en France (Dris et al., 2015), 0,32 particules.m⁻³ en Autriche (Lechner et al., 2014) ; des rejets estimés à entre 3 et

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23.10^9 particules.jour⁻¹ par les stations d'épuration dans tous les Etats-Unis (Mason et al., 2016), ou jusqu'à $7,3.10^{12}$ particules.an⁻¹ par les stations allemandes (Schmidt et al., 2020), pour un total pouvant aller jusqu'à $1,49.10^{14}$ particules.an⁻¹ sur toute l'Europe; dont jusqu'à 80 % sont transportés par les eaux fluviales jusqu'aux mers et aux océans (Komorowska-Kaufman and Marciniak, 2024). Ces particules, une fois entrées dans les milieux aquatiques, peuvent être transportées, parfois sur de longues distances, avant de sédimenter, être redéposées en milieux terrestres suite à des inondations ou les effets de marées (Vibhatabandhu et al., 2024), ou bien être consommées par le biote aquatique (Caruso et al., 2024). Une fois consommées par le biote aquatique, ces MPs peuvent remonter le long des chaînes alimentaires, et potentiellement retourner au milieu terrestre via les phénomènes de prédation à l'interface entre les milieux aquatiques et terrestres (Bartrons et al., 2018).

Les traitements des eaux aboutissent également à la concentration des MPs dans les boues d'épuration (Gao et al., 2020). Ces boues, enrichies en MPs, contenant en moyenne $3,12.10^3$ MP.g⁻¹ de boue, avec des valeurs allant de 0,193 MP.g⁻¹ de boue en Chine, jusqu'à des valeurs extrêmes de 169000 MP.g⁻¹ de boue en Norvège (Duis and Coors, 2016; Harley-Nyang et al., 2023; Vollertsen, 2017). Ces variations peuvent être expliquées par divers facteurs, passant par la taille et la densité de population du bassin versant concerné ainsi que ses caractéristiques, les conditions météorologiques, les procédés de traitement des eaux, ainsi que les méthodes de collecte et d'analyse des échantillons (Harley-Nyang et al., 2023). Les deux études aux extrêmes ont par exemple utilisé un couplage de microscopie optique et de FT-IR (spectroscopie infrarouge à transformée de Fourier), cependant les différences peuvent provenir du fait que dans l'étude avec les plus faibles concentrations, la quantification des particules a été effectuée uniquement via comptage visuel, avec la FT-IR utilisée uniquement pour caractériser le polymère, alors que dans l'étude avec les plus hautes concentrations, le comptage s'est fait via l'utilisation de la FT-IR (Vollertsen, 2017; Zhang et al., 2020).

Finalement, ces boues d'épuration sont régulièrement épandues dans les agroécosystèmes (jusqu'à 50 % en Europe et aux Etats-Unis) en tant que fertilisants organiques, provoquant un retour des MPS vers les milieux terrestres et un enrichissement en MPs des sols agricoles, avec une incorporation estimée entre 63000 et 430 000 tonnes de MPs.an⁻¹ dans les sols agricoles européens, pour un nombre de particule allant de 2,58 à

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$5,16 \cdot 10^6$ MPs.an⁻¹ selon les pays, et jusqu'à environ $1,56 \cdot 10^{14}$ MPs.an⁻¹ dans les sols agricoles chinois pour une masse de près de 79000 tonnes de MPs.an⁻¹ dans ce pays (Hatinoğlu and Sanin, 2021; Hooge et al., 2023; Milojevic and Cydzik-Kwiatkowska, 2021). La Figure 4 représente les différentes routes de contaminations des plastiques et MPs dans les environnements terrestres et aquatiques.

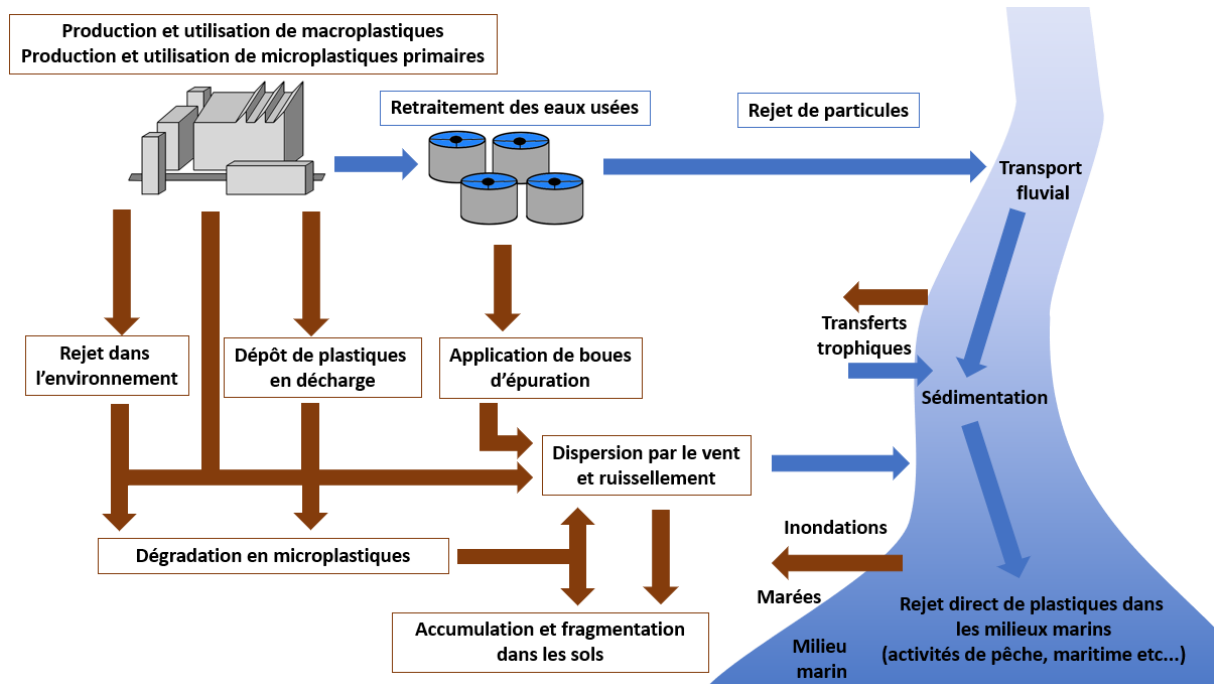


Figure 4: Origine et devenir des plastiques et MPs dans l'environnement.

III.2. Contaminations par les microplastiques

III.2.1. Contamination des écosystèmes aquatiques

Les écosystèmes aquatiques sont les milieux les plus étudiés pour ce qui concerne les contaminations en MPs. Cependant des disparités géographiques existent, par exemple, Rodrigues Dos Santos et al. (2023) ont dans leur étude estimé que 42 % des études sur les eaux de surface portaient sur l'Asie, 19 % pour l'Amérique du Nord et 10 % pour l'Amérique du Sud, 16 % portant sur l'Afrique, contre seulement 7 % pour l'Europe, ou 3 % pour l'Océanie ou l'Arctique. Ces chiffres peuvent varier en fonction des études selon les sources utilisées, mais illustrent les disparités géographiques dans les études. A titre d'exemple, les contaminations recensées des eaux de surface vont de $0,015 \text{ g.L}^{-1}$ dans des rivières belges à $0,27 \text{ g.L}^{-1}$ de MPs dans des eaux provenant de stations d'épuration des eaux anglaises, et des

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contaminations dans des lacs subalpins italiens vont de 4000 à 57000 MP.km⁻². Celles recensées en Asie vont de 0,20 MP.m⁻³ dans des lacs de haute montagne mongole à 0,92 g.L⁻¹ dans des rivières chinoises, quand les études en Amérique du Nord montrent des contaminations allant 0,043 MP.m⁻³ dans des lacs reculés aux Etats-Unis jusqu'à 1.74 g.L⁻¹ de MPs dans certains grands lacs au Canada. Les contaminations les plus importantes recensées sont cependant celles des fleuves nigériens, celles-ci pouvant aller jusqu'à 1,5 million de MP.m⁻³ (Nyaga et al., 2024).

Les écosystèmes marins sont également largement impactés par ces pollutions en MPs, passant à des contaminations de 0,0032 à 1,18 MP.m⁻³ en Antarctique ou 0,71 MP.m⁻³ au large du Qatar, ou de 70,8 MP.m⁻³ au large de l'Ecosse jusqu'à 3308 ± 2449 MP.m⁻³ au niveau des côtes de l'Afrique du Sud. Les sédiments, marins comme dulçaquicoles, sont également contaminés, allant de 1,3 à 36,3 MP.kg⁻¹ dans des sédiments côtiers russes à 2,58 MP.g⁻¹ dans des sédiments lacustres chinois, quand les sédiments côtiers européens vont de 72 à 1512 MP.kg⁻¹ (Ashrafy et al., 2023). Les interfaces entre les milieux marins et terrestres peuvent être également contaminées, avec 280 à 4816 MP.m⁻² de plage sur les littoraux atlantiques ou pacifiques (Pannetier et al., 2019). Il est à noter que les comparaisons entre études peuvent être ardues, au vue de la diversité des unités de mesures utilisées (MP.m⁻³, MP.L⁻¹, g.L⁻¹, MP.g⁻¹, MP.kg⁻¹, MP.km⁻², MP.m⁻², g.kg⁻¹...). Ceci est dû en partie à la diversité des techniques de collectes utilisées, ainsi qu'aux méthodes de quantification qui déterminent des résultats soit massiques soit en nombre de particules. Le manque de standardisation des méthodes est donc ici un frein à une comparaison efficace des contaminations en MPs des différents milieux, et donc à une bonne compréhension de ces contaminations. Une standardisation des procédés d'échantillonnage et d'analyses sera donc nécessaire à terme afin d'obtenir des résultats inter-études comparables.

III.2.2. Contamination des écosystèmes terrestres

Les contaminations en MPs des milieux terrestres, bien que moins étudiées que celles des milieux aquatiques, regroupent un certain nombre d'études. Un résumé des contaminations en MPs des milieux terrestres est présenté dans le Tableau 1. La plupart des études portant sur ces contaminations sont concentrées sur les sols agricoles (25 études sur les 31 recensées),

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la zone géographique la plus étudiée étant la Chine (19 études sur les 31 recensées). Une étude a également été réalisée sur les contaminations en MPs de 33 sols français appartenant au Réseau de Mesure de la Qualité des Sols (RMQS ; Palazot et al., 2024). Les résultats montrent une grande disparité dans les contaminations, la distribution des MPs allant de 0 MPs.kg⁻¹ dans des sols agricoles allemands à 10⁸ MPs.kg⁻¹ dans certains sols agricoles espagnols. Les sols agricoles semblent parmi les plus contaminés, même si une grande variabilité inter-étude de la distribution en MPs dans ces sols apparaît. Les résultats de l'étude sur les sols français vont dans ce sens, les sols agricoles étant largement contaminés (24 sites sur 29), contrairement aux sols de forêts (1 site sur 4), avec des contaminations moyennes allant de 60 à 1226 MPs.kg⁻¹ de sol. Ces variations peuvent être dues aux pratiques agricoles elles-mêmes (utilisation ou non de « plastic mulching » ou paillis plastique, utilisation de serre et matériel plastique ou non), ainsi que de la localisation des sites, et leur distance par rapport aux sources de pollutions plastiques. Un effet dit « effet pépite » peut également avoir lieu lors de l'échantillonnage, les MPs n'étant pas uniformément distribués dans les sols. Aussi, les contaminations des sols semblent augmenter avec l'ajout de biosolides. Ainsi, il a déjà été montré que les biosolides issus des boues d'épurations subissent un enrichissement en MPs lors du retraitement des eaux usées. L'épandage de ces biosolides sur les surfaces agricoles à des fins d'enrichissement des sols en éléments essentiels et matières organiques menant donc également à l'enrichissement de ces sols agricoles en MPs.

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Tableau 1: Synthèse des études portant sur la contamination des sols par les MPs, comportant quand fournis, la localisation de l'étude, l'utilisation du sol étudié, les paramètres des particules retrouvées, ainsi que les méthodes d'identification utilisées. N.A. : Non analysé.

Référence	Localisation	Type de sol	Profondeur	Polymère	Forme	Taille	Nb ou masse	Identification
Zhou et al., (2016)	Hebei, Chine	Sols côtiers	2 cm	N.A.	Fragments, granules, fibres et films	0,12 - 4,67 mm	634 MP/kg	Microscope (SEM)
Li et al., (2019)	Jiangsu, Chine	Sols agricoles	N.A.	PP, PE, PET, PAN	Fibres, fragments	0,02 - 0,25 mm	420 - 1290 MP/kg	Microscope (optique) et μ FT-IR
Liu et al., (2018)	Shangai, Chine	Sols agricoles	3 - 6 cm	PP, PE, PET	Fibres, fragments, films	0,03 - 5 mm	62,5 - 78 MP/kg	Microscope (optique) et μ FT-IR
Zhang et al., (2018)	Nord-Ouest de la Chine	Sols agricoles (maraichage)	0 - 30 cm	LDPE, PP	N.A.	> 0,1 mm	0,46 - 0,54 MP/kg	Microscope
Zhang and Liu, (2018)	Yunnan, Chine	Sols de serres et zone tampon boisée	5-10 cm	N.A.	Fibres, films et fragments	0,05 - 1 mm	7100 - 42960 MP/kg	Microscope (optique)
Zhou et al., (2018)	Shandong, Chine	Sols côtiers	2 cm	PE, PP, PS, PUR	Mousses, paillettes, fragments et fibres	~5 mm	1,3 - 14713 MP/kg	Microscope (optique, SEM) et FT-IR
Lv et al., (2019)	Shanghai, Chine	Sols agricoles (rizière)	10 cm	PE, PP, PVC	Fibres, granules, fragments et films	0,02 - 1 mm	10.36 MP/kg	Microscope (optique) et μ -FT-IR
Zhou et al., (2019)	Wuhan, Chine	Sols agricoles (maraichage)	5 cm	PE, PP, PS, PA, PVC	Fibres, fragments, films, mousses et billes	< 0,1 mm	2230 - 69300 MP/kg	Microscope (optique, SEM) et spectroscopie Raman

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Référence	Localisation	Type de sol	Profondeur	Polymère	Forme	Taille	Nb ou masse	Identification
Chen et al., (2020)	Wuhan, Chine	Sols agricoles (maraichage)	5 cm	PA, PP	Microbilles, fragments, mousses et fibres	< 0,2 mm	320 - 12560 MP/kg	Microscope (optique) et spectroscopie micro-Raman
Ding et al., (2020)	Shaanxi, Chine	Sols agricoles	10 cm	PS, PE, PP, PVC, PET	Fibres, films, particules, fragments	0 - 0,5 mm	1430 - 3410 MP/kg	Microscope (optique) et μ FT-IR
Huang et al., (2020)	Xinjiang, Chine	Sols agricoles (coton)	0 - 40 cm	N.A.	Films et fragments	~2 mm	80 - 1076 MP/kg	Microscopie (optique, SEM) et FT-IR
Zhou et al., (2020)	Baie d'Hangzhou, Chine	Sols agricoles (paillage plastique)	10 cm	N.A.	Fragments, fibres et films	1-3 mm	571 MP/kg	Microscope et FT-IR
Huang et al., (2021)	Yunnan, Chine	Sols agricoles (sans paillage) Sols agricoles	0 - 30 cm	N.A.	Fibres, fragments	<0,1 - 5 mm	263 MP/kg 900 - 40800 MP/kg	N.A.
L. Wang et al., (2022)	Chine	Sols agricoles	N.A.	PE, PA	Fibres, fragments, films et sphères	0,02 - 5 mm	2783 - 6366 MP/kg	Microscope (optique) et μ FT-IR
Yu et al., (2021)	Shandong, Chine	Sols agricoles	0 - 5 cm	PP, PE, PS, PUR	Fragments, films, fibres, granules	<0,5 - 5 mm	310 - 5698 MP/kg	Microscope (optique) et μ FT-IR
L. Chen et al., (2022)	Chine	Sols agricoles	N.A.	PP, PE, PS, copolymères	Films, fibres, granules, mousses	<0,05 - 5 mm	2462 \pm 3767	N.A.
Lang et al., (2022)	Qinghai, Chine	Sols agricoles	0 - 10 cm	N.A.	Films et fibres	<0,5 - 5 mm	240 - 3660 MP/kg	Microscopie (optique)
(J. Li et al., 2022)	Xinjiang, Chine	Sols agricoles	0 - 10 cm	PE, PP, PS, PVC	Fragment, fibres, films, mousses	<0,5 - 5 mm	288 - 1452 MP/kg	Microscope (optique) et FT-IR

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Référence	Localisation	Type de sol	Profondeur	Polymère	Forme	Taille	Nb ou masse	Identification
Wenfeng Li et al., (2023)	Xinjiang, Chine	Sols agricoles	0 - 30 cm	PE, PP, PS	Films, mousses, fibres	0,05 - 5 mm	3178 ± 3172 (maraichage) - 7763 ± 2773 (serres) MP/kg	Microscope (optique) et FT-IR
Mohamed Nor and Obbard, (2014)	Singapour	Sols de mangroves	3 - 4 cm	PE, PP, PA et PVC	Fibres, particules	< 0,02 mm	12 - 63 MP/kg	Microscope (optique) et FT-IR
S.-K. Kim et al., (2021)	Yongin, Corée	Sols agricoles (rizière, paillage et serres)	5 cm	PE	Fragments, fibres et feuilles	0,1-0,3 mm	130 - 563 MP/kg	Microscope (optique) et FT-IR
Piehl et al., (2018)	Franconie, Allemagne	Sols agricoles	5 cm	PE, PS, PP, PVC, PET, PMMA	Films, fragments et fibres	1-5 mm	0 - 1,25 MP/kg	Microscope (optique) et FT-IR
Harms et al., (2021)	Schleswig-Holstein, Allemagne	Sols agricoles	0 - 30 cm	PE, PP, PA	Films, fragments et paillettes	1 - 5 mm	0 - 217 MP/kg	Microscope (optique) et FT-IR
Scheurer and Bigalke, (2018)	Suisse	Sols de zones inondables	5 cm	PE, PS, PA, PVC, PP, SBR	N.A.	0,125 - 0,5 mm	< 593 MP/kg	FT-IR et spectroscopie Raman
Edo et al., (2020)	Madrid, Espagne	Sols agricole (avec biosolides)	N.A.	PE, PP, PES, acryliques	Fragments, fibres	0,03 - 2,2 mm	302000 MP/kg	Microscope (optique) et FT-IR
Van Den Berg et al., (2020)	Valence, Espagne	Sols agricoles (avec biosolides) Sols agricoles (sans biosolides)	10 - 30 cm	PP, PVC	Fragments, films et fibres	0,15 - 0,25 mm	2,13.10 ⁶ - 3,06.10 ⁸ MP/kg 9,3.10 ⁶ - 1,1.10 ⁷ MP/kg	Microscope (optique) et μ-FT-IR
Crossman et al., (2020)	Ontario, Canada	Sols agricoles (avec biosolides) Sols agricoles (sans biosolides)	0 - 15 cm	PE, PP, PES, acryliques	Fibres, fragments	0,1 - 1 mm	18 - 541 MP/kg 25 - 298 MP/kg	Microscope (optique) et FT-IR

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Référence	Localisation	Type de sol	Profondeur	Polymère	Forme	Taille	Nb ou masse	Identification
Corradini et al., (2021)	Santiago, Chili	Sols agricoles (maraichage, pâture, prairies)	N.A.	PE, PP, PS, PUR, Acrylates	Fibres, films, fragments, granules	0,076 mm ² (moyenne)	540 MP/kg (maraichage), 420 MP/kg (pâture), < 200MP/kg (autres)	Microscope (optique) et FT-IR
Corradini et al., (2019)	Mellipilla, Chili	Sols agricoles	25 cm	Acrylates, PUR, PE, PEVA, PP, PS, PES, PA, PLA	Fibres, films, fragments et granulés	~1 mm	600 - 10400 MP/kg	Microscope (optique)
Helcoski et al., (2020)	Washington DC, Etats-Unis	Sols de zone humide	5 cm	PS, PE, PP, PET, caoutchoucs	Fragments, fibres	0,075 - 5 mm	1270 MP/kg	Microscope (optique) et FT-IR
Fuller and Gautam, (2016)	Sydney, Australie	Sols en zones industrielles	N.A.	PVC, PE, PS	N.A.	N.A.	300 - 67500 mg/kg	FT-IR
Palazot et al., (2024)	France	Sols forestiers, sols agricoles (maraichage, vigne, prairies)	0 – 20 cm	PE, PP, PS, PVC, PET, PU	N.A.	315 – 5000 µm	65 – 1226 MP/kg	ATR-FT-IR

Tout comme pour les milieux marins, les comparaisons inter-études dans les milieux terrestres peuvent poser problème, les résultats pouvant être exprimés en nombre ou en masse de MPs par quantité de sol. De plus, une grande disparité en termes de résultats apparaît. Elle provient en premier lieu des différences de contamination entre les différents milieux et les différents échantillons. Cependant, une part de cette variation provient également des procédures utilisées pour la quantification des MPs dans les milieux, qu'ils soient biotiques ou abiotiques. En effet, aucune procédure analytique complète standardisée n'existe pour la quantification des MPs dans les différentes matrices. Ainsi, différentes méthodes analytiques peuvent donner des résultats différents pour des niveaux de contamination similaires.

III.2.3. Techniques analytiques pour l'étude des microplastiques

De par la composition complexe des milieux contaminés par les MPs (notamment les sols et les matrices biologiques), différentes étapes sont nécessaires afin de pouvoir analyser les MPs depuis ces matrices (récupération, extraction et analyse). Ici, l'étape pouvant être la plus complexe est l'étape d'extraction des MPs depuis les différentes matrices, qui réclame des processus de digestion des matrices ou de séparation des MPs. Ces procédures analytiques ne sont pas standardisées. Aussi, différentes techniques sont employées dans ce cadre, tant au niveau de l'échantillonnage des matrices, que de l'extraction ou l'analyse des MPs. Les problématiques liées à l'échantillonnage des matrices environnementales ne sont pas spécifiques à l'étude des MPs et ne seront pas détaillées ici. Cette partie référencera brièvement les principales techniques décrites sur la base de différentes revues, pour la purification et l'analyse des MPs (He and Luo, 2020; Issaka et al., 2023; Mushtak et al., 2024; Yan and Yang, 2023; Z. Zhang et al., 2022b).

III.2.3.1. Techniques pour l'extraction des microplastiques

Généralement, après échantillonnage d'une matrice environnementale ou biologique, la première étape consiste au séchage et, si besoin, au tamisage de l'échantillon. Les températures de séchage sont comprises entre 40 et 70°C (étape principalement utilisée pour les échantillons environnementaux solides, *i.e.* sols ou sédiments). Ces températures permettent d'obtenir un échantillon sec pour les étapes suivantes, tout en évitant une dénaturation voire une destruction thermique des possibles MPs contenus dans les échantillons. Suite au séchage, les échantillons sont traditionnellement tamisés avec une taille

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de maille de 5 ou 2 mm, avec parfois l'utilisation de tamisage séquentiel, avec des tailles de mailles allant de 5 à 1 mm (Thomas et al., 2020), afin de faciliter l'analyse à suivre sur les différentes tailles de particules. Suite à cette première étape de préparation vient l'étape d'extraction des MPs, qui peut se faire de différentes manières :

- La séparation par la densité, qui utilise la différence de densité entre les MPs et les particules de sol pour séparer les MPs mis en solution. Les principales solutions utilisées sont des solutions saturées de NaCl ($d=1,2 \text{ g.cm}^3$), de polytungstate de sodium ($d=1,4 \text{ g.cm}^3$), de CaCl_2 ($d=1,3-1,5 \text{ g.cm}^3$), de NaBr ($d=1,55 \text{ g.cm}^3$), de ZnCl_2 ($d=1,6 \text{ g.cm}^3$), de ZnBr_2 ($d= 1,7 \text{ g.cm}^3$) ou de NaI ($d=1,8 \text{ g.cm}^3$). Le choix de ces solutions dépend de la densité des MPs à extraire, du coût de la solution, ainsi que de sa potentielle toxicité (Tirkey and Upadhyay, 2021).

- La flottation par moussage, méthode basée sur l'adsorption spécifique de bulles générées dans un milieu aqueux liée à la nature hydrophobe des MPs, leur permettant de remonter à la surface du milieu et donc les séparant du milieu environnant.

- L'extraction magnétique, méthode utilisant des nanoparticules de fer qui seront liées aux MPs, permettant de les extraire via l'utilisation de champs magnétiques. Cette méthode permet une bonne extraction des MPs, mais nécessite néanmoins de fonctionnaliser les nanoparticules de fer auparavant. De plus, l'efficacité de la méthode varie avec la taille des particules et le pH du milieu aqueux dans lequel se passe l'extraction (Grbic et al., 2019).

- La séparation dans l'huile, qui exploite la lipophilie et l'hydrophobie des MPs, qui sont extraits dans une colonne de séparation sous l'effet de la gravité quand les impuretés sont retenues dans le milieu aqueux ou sur la phase solide. Les huiles utilisées pour cette méthode de séparation sont l'huile de canola, l'huile de castor et l'huile d'olive. Cependant, cette méthode limite la taille extractible des MPs, et les colonnes de séparation ont tendance à rapidement se boucher. De plus, les huiles interfèrent avec les techniques d'identification des MPs, ceux-ci devant par la suite être rincés à l'hexane avant identification (Tirkey and Upadhyay, 2021).

- La séparation électrostatique, où le principe est d'utiliser un séparateur électrostatique afin de séparer les particules de sol plus conductrices, pour garder les MPs moins conducteurs. Cependant cette méthode est assez peu efficace pour la récupération des plus petits MPs

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(sous 60 μm), ou la récupération de MPs dans des échantillons avec de hautes teneurs en limons et/ou en argiles (Enders et al., 2020).

Suite à l'étape d'extraction (pour les échantillons environnementaux) ou précédant celle-ci (pour les échantillons biologiques), une étape de digestion de la matière organique est généralement nécessaire afin de permettre une analyse non-biaisée des MPs. Différentes méthodes de digestion de la matière organique sont actuellement disponibles (Enders et al., 2017; Prata et al., 2019):

- L'oxydation à l' H_2O_2 , le plus communément à 30 % v/v à chaud (65 – 70°C), qui entraîne l'oxydation et la décomposition de la matière organique. Cette méthode est efficace pour l'élimination de la matière organique, mais elle a tendance à partiellement dégrader voire détruire les MPs, selon leur nature polymérique. Ce phénomène peut soit mener à l'apparition d'artéfacts dans les résultats d'analyse, soit mener à des analyses négatives suite à la destruction des MPs. Aussi d'autres méthodes peuvent être à privilégier.

- Les digestions acides et alcalines, à base d' HCl , de HNO_3 ou de HClO_4 pour la digestion acide, et de NaOH ou de KOH pour la digestion alcaline, qui se font à chaud (environ 70°C). Ces méthodes se basent sur la digestion ou la saponification de la matière organique, et sont très efficaces pour son élimination. Cependant, ces méthodes sont également susceptibles de détruire les MPs (notamment pour les digestions acides) ou de les dégrader en surface, ce qui peut interférer avec les analyses ultérieures.

- La digestion par le réactif de Fenton, méthode basée sur l'oxydation de la matière organique par le peroxyde d'hydrogène, mais avec adjonction de fer comme catalyseur de la réaction. Cette méthode est très efficace pour la purification des MPs, et est moins agressive que l'oxydation par l' H_2O_2 , cependant des précautions doivent être prises (température de réaction sous 40°C), cette réaction pouvant être très exothermique et donc dégrader les MPs.

- La digestion enzymatique, se basant sur l'utilisation de protéases, de cellulases et de chitinases, combinées à une incubation préliminaire dans du SDS et de l' H_2O_2 . Cette méthode de purification est douce et efficace dans l'élimination de la matière organique. Cependant elle nécessite un design spécifique d'enzymes pour chaque type d'échantillon d'eau, de sol, de sédiment ou biologique, pour prendre en compte les spécificités de chaque échantillon. De

plus, les MPs issus de bioplastiques peuvent également être dégradés par cette digestion enzymatique.

Les méthodes d'extraction et de purification des MPs présentent toutes des avantages et des inconvénients. Le choix parmi ces différentes méthodes est donc à déterminer à la fois en fonction de la nature des échantillons et de la nature des MPs présents dans les échantillons. Un tel choix peut-être difficile, notamment concernant la purification des MPs depuis les échantillons, chacune des méthodes impliquant un risque de détérioration des MPs. Un tel choix se complique d'avantage lors du traitement d'échantillons environnementaux, lorsque l'on ne connaît pas a priori la nature polymérique des MPs présents.

III.2.3.2. Techniques de détection et quantification des microplastiques

Selon les objectifs fixés par l'étude, différents types d'analyses qualitatives et/ou quantitatives des MPs dans les sols ou les tissus peuvent être employées. Ces techniques dépendent du temps et du budget alloué à l'analyse, ainsi que de l'aspect purement qualitatif ou quantitatif de l'étude (He and Luo, 2020; Issaka et al., 2023; Mushtak et al., 2024; Watteau et al., 2018; Yan and Yang, 2023; Z. Zhang et al., 2022b) :

- L'identification visuelle, la méthode la plus simple et la moins onéreuse. Cette méthode permet de déterminer la taille, la forme et la couleur à l'aide d'un microscope optique. Toutefois cette méthode est très chronophage, et le taux d'erreur peut osciller entre 20 et 70 % étant donné la difficulté de déterminer visuellement si une particule est un MP ou non, notamment quand ceux-ci ne sont pas colorés. Pour pallier a ceci, différentes méthodes ont été développées, comme la coloration au rouge de Nile (pour colorer les MPs) ou alors la méthode dite de la seringue chaude (comparaison photographique de particules avant et après apposition d'une pointe de seringue chaude, entre 3 et 5 secondes à 130°C, afin de déterminer la présence ou non de déformation plastique sous l'effet de la chaleur). La détection visuelle par microscopie optique pose également le problème de la détection des plus petites particules. Pour pallier a cela, une seconde phase de détection par microscopie électronique à balayage peut être envisagée pour détecter ces MPs de petite taille. Cependant, de telles analyses sont longues, complexes à mettre en œuvre et coûteuses.

- La spectroscopie vibrationnelle, comprenant la spectroscopie infrarouge à transformée de Fourier (FT-IR) et la spectroscopie Raman, qui sont deux méthodes non destructives. La FT-

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IR se base sur l'absorption en surface de l'objet de différentes longueurs d'ondes infrarouges (spectroscopie diffractive), qui forment un spectre d'absorption infrarouge permettant de déterminer les caractéristiques moléculaires de la surface de l'objet. La spectroscopie Raman elle, envoie un faisceau de lumière monochromatique sur l'échantillon, et analyse les décalages de fréquence de la lumière diffusée afin de définir les caractéristiques moléculaires de surface de l'échantillon. Ces deux méthodes permettent ensuite de comparer les spectres d'absorption ou de diffusion à des bases de données afin de déterminer la nature de la particule scannée. Ces deux méthodes sont considérées comme étant les plus fiables concernant la détection des MPs, et peuvent permettre la détection de particules jusqu'à 20 μm (FT-IR) à 1 μm (Raman), via les méthodes appelées micro-FT-IR ou micro-Raman. Cependant, ce sont des méthodes chronophages et coûteuses, et elles sont susceptibles à la présence d'impuretés et de matière organique résiduelle dans les échantillons.

- L'analyse thermique, comprenant la pyrolyse couplée à la chromatographie en phase gazeuse-spectrométrie de masse (py-GC-MS), la thermodésorption couplée à la chromatographie en phase gazeuse-spectrométrie de masse (TED-GC-MS), la thermogravimétrie (TGA) et la calorimétrie différentielle à balayage (DSC). Ces techniques se basent soit sur les changements des propriétés physicochimiques des MPs en fonction de la température, soit sur l'analyse de l'émission de produits de dégradation volatils des MPs à haute température. Bien que ces techniques ne nécessitent pas de pré-traitement spécifique et peuvent aider à l'identification de la composition chimique des polymères employés dans les MPs ainsi que de leurs additifs, elles subissent une limitation concernant la taille de l'échantillon analysable (difficultés d'analyse pour des MPs sous 100 μm), et les limites de détection des molécules servant de marqueurs des microplastiques (de l'ordre du μg ; Gregoris et al., 2023) ce qui limite l'analyse d'échantillons de faible masse. De plus, ces techniques peuvent être sujettes à l'erreur pour des MPs ayant des températures de dégradations thermiques proches. De plus ces analyses sont des méthodes destructives, et ne permettent donc pas de réutiliser les échantillons pour des analyses ultérieures.

- Pour contourner les verrous techniques liés aux techniques de quantification directes des MPs, des méthodes de quantification indirectes peuvent être utilisées. Ces méthodes sont principalement basées sur la technique du marquage élémental (exemple : marquage des particules au gadolinium ; Abdolahpur Monikh et al., 2022) des MPs et dosage de ces éléments

dans les échantillons. Ces techniques seront donc développées au cours de ce travail de thèse. Cependant, l'applicabilité de ces méthodes reste encore limitée au travail nécessitant une contamination *ex situ*, en laboratoire, des échantillons environnementaux ou biologiques, les particules nécessitant d'être marquées avant utilisation.

Ces chapitres nous ont permis de voir que les plastiques sont très largement produits et utilisés dans la plupart des activités humaines. Une fois les plastiques utilisés et rejetés, ils peuvent contaminer tous les environnements, terrestres, comme aquatiques ou aériens. Les MPs, suite au vieillissement et au fractionnement des plastiques, peuvent à leur tour contaminer très largement tous les écosystèmes. Cependant, la détection et la quantification des MPs dans les milieux, notamment dans les matrices complexes telles que les sols et les matrices biologiques présentent encore un certain nombre de verrous méthodologiques en terme de détection directe. Les méthodes sont chronophages, coûteuses, et peuvent être sujettes à l'erreur. Ceci est dû à la fois à la complexité des matrices contaminées, à la nature particulière des MPs, ainsi qu'à leurs propriétés physicochimiques qui peuvent être relativement semblables. Ces difficultés font qu'aucune méthode n'est actuellement standardisée pour la détection et la quantification directe des MPs. Pour compenser les problématiques liées à la quantification directe, des méthodes de quantifications indirectes peuvent-être envisagées, comme l'utilisation de particules marquées. Cependant, ces méthodes concernent principalement les études *ex situ* en conditions contrôlées, et sont difficilement applicables à l'étude des contaminations environnementales des MPs.

IV. Ecotoxicité des microplastiques

La contamination en MPs des milieux est globale, et malgré les problématiques liées aux techniques analytiques actuelles, on peut noter que les sols sont particulièrement touchés. L'enrichissement des sols ne concerne pas que les sols agricoles. En effet, tous les types de sols sont touchés, montrant la capacité de dispersion à longue distance des MPs soit par l'eau, soit par l'air. Finalement, une fois dans l'environnement, ces MPs présentent donc un risque de contamination du biote via différentes voies. Ces voies peuvent être soit orale, via l'ingestion de sols ou d'items alimentaires contaminés par les MPs, soit pulmonaire à travers

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l'inhalation d'air contenant des MPs. Une dernière voie d'exposition peut être par diffusion cutanéomuqueuse pour des organismes vivant au contact ou à l'intérieur de milieux contaminés par les MPs, à condition que les MPs puissent être de tailles suffisamment petites pour traverser la barrière cutanéomuqueuse. Les voies décrites précédemment prévalent pour les animaux, cependant les plantes et les microorganismes peuvent également être exposés, via une internalisation racinaire pour les plantes, ou via contact pour les microorganismes (voire une internalisation pour les plus petits MPs).

La contamination des organismes du sol par les MPs peut entraîner des effets écotoxiques sur ces organismes. Cette toxicité des microplastiques est influencée par différents paramètres, comme le type de polymère (la toxicité pouvant dépendre de la nature du polymère) et la concentration d'exposition (une concentration plus élevée pouvant donner une toxicité plus forte). La taille et la forme des MPs sont aussi importantes, des particules plus petites pouvant pénétrer plus en profondeur dans les tissus et pouvant donc exercer une toxicité plus élevée, quand des MPs de formes irrégulières peuvent plus facilement léser les tissus par une action mécanique, et donc augmenter l'inflammation de ceux-ci. La charge de surface des MPs joue aussi un rôle, une charge de surface plus importante permettant aux MPs d'interagir plus facilement avec les membranes cellulaires, et plus largement avec les biomolécules (Choi et al., 2021; Ma et al., 2020).

Les MPs peuvent également adsorber d'autres xénobiotiques, comme des éléments traces métalliques (ETM) ou des polluants organiques (PO) présents de façon concomitante aux MPs dans l'environnement, qui pourront potentiellement être désorbés dans les organismes après ingestion des MPs, et ainsi exercer des effets toxiques, ceux-ci pouvant s'additionner ou se potentialiser avec les effets des MPs. De plus, dans les plastiques, sont adjoints aux polymères des additifs, eux-mêmes étant des ETM ou des PO, qui sont ajoutés dans la matrice polymérique ou à sa surface pour en améliorer les caractéristiques à l'utilisation du produit final. Ces additifs peuvent avoir des effets toxiques pour le biote terrestre (Catrouillet et al., 2021; Y. Li et al., 2023; J.-L. Xu et al., 2022).

Les MPs, une fois dans l'environnement, peuvent aussi (si leur taille est assez grande) être colonisés par des microorganismes, formant alors un micro-habitat spécifique, aussi appelé plastisphère. Les MPs peuvent alors agir comme vecteur de dissémination de microorganismes potentiellement pathogènes, mais peuvent aussi permettre un échange

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horizontal de gènes de résistance aux antibiotiques (Sun et al., 2022). Enfin, l'état de vieillissement des MPs a aussi une importance, car le vieillissement des particules peut à la fois changer leur forme, leur charge de surface, ainsi que le nombre et le type de fonctions de surface. Ce vieillissement induit donc des changements à la fois dans la capacité des MPs à exercer des effets toxiques, ainsi que dans leur capacité à adsorber d'autres contaminants et à être colonisés par des microorganismes (Liu et al., 2020; Rai et al., 2022).

Les prochaines parties traiteront de la toxicité des MPs sur le biote. Il est cependant à noter que l'évaluation de la toxicité des MPs sur la faune terrestre est assez peu représentée dans les études, celles-ci ne correspondant qu'à environ 8 % des études (Bank, 2022), le reste se portant sur les milieux aquatiques (Figure 5). Le contraste entre les contaminations importantes des milieux terrestres et le nombre d'études s'intéressant à leurs effets se doit d'être adressé. Ce travail de thèse se concentrera donc sur la toxicité des MPs sur la faune terrestre. Aussi, les prochains chapitres traiteront donc des effets des MPs sur cette faune terrestre (invertébrés et vertébrés).

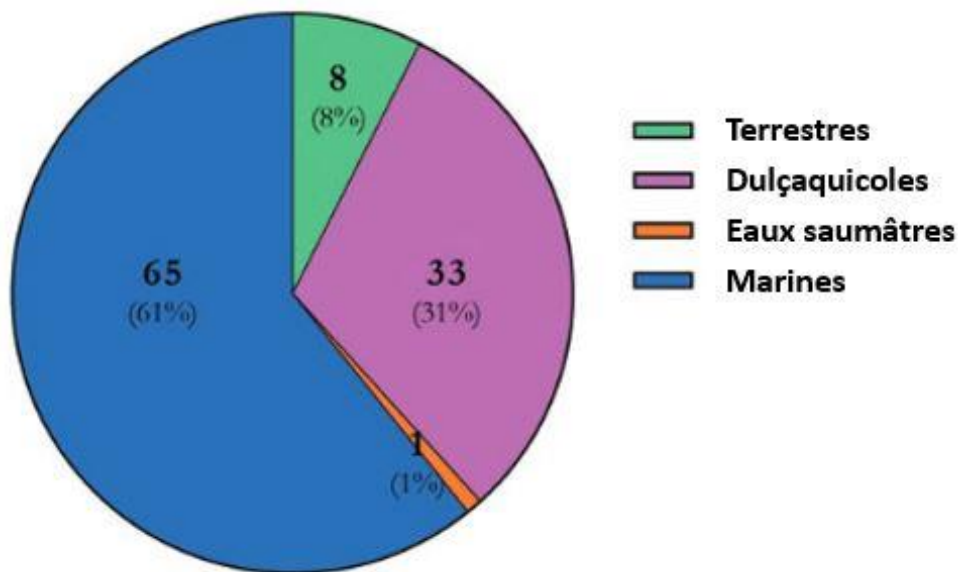


Figure 5: Pourcentage d'espèces des différents compartiments environnementaux utilisées dans les études sur l'écotoxicité des MPs sur la faune. Adapté de Bank, (2022)

IV.1. Ecotoxicité des MPs sur les organismes terrestres

La toxicité des MPs envers le biote est le paramètre portant sur les impacts associés aux MPs pour le biote terrestre le plus étudié. Les études présentées ici sont synthétisées dans les Tableaux 2 et 3, qui référencent les travaux portant sur les effets des MPs sur les invertébrés terrestres (Tableau 2) et sur les vertébrés terrestres (Tableau 3) respectivement.

Les études concernant les invertébrés terrestres présentées ici couvrent différents modèles biologiques, le plus étudié étant les vers de terre (15 études sur 3 espèces), suivi par les nématodes (9 études sur 1 espèce), puis les escargots (3 études sur 2 espèces), les cloportes (2 études), les modèles collemboles et enchytrés (3 études), coléoptères et acariens (1 étude chacun). Deux études se penchent aussi sur les effets des MPs sur des communautés d'arthropodes et de microarthropodes mais sans citer spécifiquement les espèces retrouvées.

Chez les vers de terre, les polymères étudiés ont été le PE, le PS, le PLA, le PP, les fibres de polyester (PES) et le copolymère de polypropylène (PPC), pour des durées d'exposition allant de 14 à 180 jours. Les indicateurs influencés par les MPs sont la survie (diminution de survie ou pas d'effet pour le PS, à des tailles de 0,1 – 1 ou 25 – 30 μm , respectivement, diminution de survie induite par le PP ; Jiang et al., 2020 ; Kwak & An, 2022 ; Tang et al., 2023), la croissance (stimulation à faible concentration en PS ou diminution à forte concentration en PS, PE, PLA ou PPC; Ding et al., 2021 ; Huerta Lwanga et al., 2016 ; Kwak & An, 2021), la reproduction (inhibition de reproduction par le PS, le PE, le PLA ou le PPC ; Ding et al., 2021 ; Holzinger et al., 2022 ; Rodriguez-Seijo et al., 2017 ; Sobhani et al., 2022), le stress oxydatif (inhibition de l'activité estérase par le PE et induction de stress oxydatif par le PE, le PS et les fibres de PES ; Holzinger et al., 2022 ; Prendergast-Miller et al., 2019 ; Rodriguez-Seijo et al., 2018), la neurotoxicité (induction de neurotoxicité par le PE et le PLA) et les dommages tissulaires (induction de lésions histopathologiques intestinales en présence de PP, de PE ou de PS, altérations de l'appareil reproducteur induit par le PS ; K.Chen et al., 2022 ; T.Li et al., 2022). Deux études sur les vers de terre ont également évalué les contaminations des animaux en MPs en utilisant la microscopie à fluorescence, avec des concentrations moyennes en MPs quantifiées allant de $0,015 \pm 0,001$ à $0,094 \pm 0,003 \mu\text{g PS.mg}^{-1}$ tissu (Jiang et al., 2020) pour la première étude, la deuxième (Xiao et al., 2022) fournissant des données d'intensité de fluorescence sans données quantifiées concernant les concentrations en MPs.

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Chez les nématodes, les MPs tels que le PS et le PE peuvent engendrer une diminution de la durée de vie, une inhibition de croissance et des capacités de reproduction, ainsi que de la fonction de locomotion associée à une neurotoxicité, et une perturbation du métabolisme lipidique et des dommages à l'ADN pour des concentrations de l'ordre du μg au mg.L^{-1} en expérimentation *ex situ* (H.Chen et al., 2022 ; Fueser et al., 2022 ; Kim et al., 2020 ; Lei et al., 2018 ; Schöpfer et al., 2020 ; Shang et al., 2020 ; Y.Yu et al., 2020 ; Yu et al., 2023). L'étude *in situ* des effets du LDPE de 5 à 15 g.m^{-2} a elle montré une diminution de l'abondance de certaines lignées de nématodes (à l'exception des bactériophages et mycophages ; Selonen et al., 2021).

Les études portant sur les escargots (*Achatina reticulata* et *Achatina fulica*) ont montré, suite à une exposition au PET, une stimulation de la croissance chez *A.reticulata* (PET 1 – 10 % m/m, 50 à 1000 μm ; De Felice et al., 2021) mais une baisse de la consommation d'item alimentaire chez *A.fulica* (PET 14 – 710 mg.kg^{-1} , fibre de 76 x 1250 μm) accompagnée par une augmentation du stress oxydatif et des dommages tissulaires intestinaux (Song et al., 2019). L'étude portant sur *A.fulica* nourrie sur des blocs de polystyrène expansé (EPS) a elle montré des changements dans la composition de leur communauté commensale intestinale suite à cette exposition (Song et al., 2020). Les deux études sur *A.fulica* ont également étudié le contenu intestinal des escargots par microscopie, et ont observé des MPs dans l'intestin des animaux. Elles n'ont cependant pas pu quantifier cette contamination dans les tissus.

Les études portant sur l'espèce de cloportes *Porcellio scaber* n'ont pas montré d'effet du PE sur la consommation alimentaire ou les réserves énergétiques de l'animal lors d'une exposition de 14 jours (Jemec Kokalj et al., 2018), cependant leur viabilité hémocytaire a été diminuée après 21 jours d'exposition au PP, et cette exposition a engendré une réponse immunitaire (augmentation des granulocytes) de la part de l'animal (Jemec Kokalj et al., 2022). Le PP a également provoqué après 21 jours d'exposition une perturbation de l'activité énergétique chez le coléoptère *Tenebrio molitor*, mais n'a pas eu d'effet sur la reproduction d'*Enchytraeus crypticus* (Jemec Kokalj et al., 2022). Les études sur les collemboles ont elles montré que le PE (Kim & An, 2020) et le PVC (Zhou et al., 2018) entraînent une perturbation de leur microbiote intestinal, ainsi qu'une diminution de leur masse (PE) et une perte de motilité (PVC).

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Enfin, les études se portant à l'échelle des communautés montrent que le LDPE pouvait induire une diminution ou une augmentation de l'abondance en microarthropodes pour des concentrations de 5 à 15 g.m⁻² (Huang et al., 2023 ; Lin et al., 2020).

Les effets peuvent donc varier selon les conditions expérimentales. Plus précisément, les effets des MPs sur le sol et la faune terrestre varient en fonction de différents facteurs, tels que la taille, l'hydrophobicité, la charge, la densité ou la forme des MPs (De Souza Machado et al., 2020). Les concentrations d'exposition, l'espèce considérée, ainsi que les relations entre les MPs et la matrice (sol, nourriture, ... pouvant influencer la capacité des MPs à être ingérés par les organismes, ou leur toxicité une fois ingérés) sont également à prendre en considération.

Les effets présentés par ce corpus d'études montrent une certaine variabilité selon le polymère considéré, sa taille, sa concentration d'exposition, ainsi que l'espèce considérée. Malgré des variations dans l'étendue des effets, avec parfois des résultats contradictoires (diminution ou augmentation de l'abondance en microarthropodes, diminution ou non de la survie chez le ver de terre), une tendance générale se dégage. Prises dans leur ensemble, ces études montrent une tendance des MPs à exercer des effets toxiques sur les invertébrés terrestres, tant sur des marqueurs individuels (survie, croissance, reproduction, locomotion), sub-individuels (perturbation métabolique, stress oxydatif, neurotoxicité, lésions tissulaires), que populationnels et communautaires.

Cependant, la diversité de macro-invertébrés étudiée est relativement faible au vu du nombre d'espèces potentiellement impactées, ce qui représente une lacune dans les recherches, en particulier concernant les invertébrés situés à l'interface sol-plante-atmosphère, qui intègrent diverses sources et voies de contamination. Parmi ces macro-invertébrés, les escargots sont très peu représentés, pourtant la présence de MPs dans leurs tissus est avérée (Panebianco et al., 2019; Rodrigues et al., 2023). De plus, les études se penchant sur les effets à long terme des MPs sur les macro-invertébrés du sol sont également peu représentées. Cette thèse traitera donc des effets sub-chroniques et chroniques des MPs sur l'escargot.

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Tableau 2: Référencement des études portant sur la toxicité directe des MPs sur les invertébrés terrestres. N.A. : Non applicable.

Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Source d'exposition	Durée d'exposition (jours)	Effets
Huerta Lwanga et al., (2016)	<i>Lumbricus terrestris</i>	PE	< 150	7 – 60 % (m/m)	Sol	60	<ul style="list-style-type: none"> ↘ Croissance ↗ Mortalité
Prendergast-Miller et al., (2019)	<i>Lumbricus terrestris</i>	PES (fibres)	41 x 363	0,1 ; 1 % (m/m)	Sol	35	<ul style="list-style-type: none"> ↗ Expression mt-2 et ↘ Expression hsp70 (signe de stress)
Kwak and An, (2021)	<i>Eisenia andreei</i>	PE	180-212 et 250-300	1 g/kg	Sol	21	<ul style="list-style-type: none"> ↘ activité estérase dans coelomocyte ↘ spermatogénèse ↗ Altérations tissulaires dans appareil reproducteur (mâle)
Rodriguez-Seijo et al., (2017)	<i>Eisenia andreei</i>	LDPE	250 - 1000	62,5; 125; 250; 500; 1000 mg/kg	Sol	28 (toxicité), 56 (reproduction)	<ul style="list-style-type: none"> ↗ Lésions histopathologiques et inflammation intestinale
Sobhani et al., (2022)	<i>Eisenia fetida</i>	PE	250 - 1000	0,01 ; 0,1 ; 0,5 %	Sol	28 (F0) puis 180 (F1)	<ul style="list-style-type: none"> Relargage et bioaccumulation de phtalates depuis PE ↗ Dommages à l'ADN (F0 et F1) ↘ Reproduction (F0 et F1)
Rodríguez-Seijo et al., (2018)	<i>Eisenia fetida</i>	LDPE	250 - 1000	62,5; 125; 250; 500; 1000 mg/kg	Sol	28	<ul style="list-style-type: none"> ↗ Stress oxydatif

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Source d'exposition	Durée d'exposition (jours)	Effets
K. Chen et al., (2022)	<i>Eisenia fetida</i>	PE ; PP	180 - 250	0,02 % m/m	Sol	14	<ul style="list-style-type: none"> ↗ Lésions intestinales Perturbations osmorégulation Perturbation transcriptome lié à appareil digestif et système immunitaire
Kwak and An, (2022)	<i>Eisenia andreii</i>	PP (fibres)	191 x 15 ; 891 x 15	1000 mg/kg	Sol	21	<ul style="list-style-type: none"> ↘ Survie ↘ Viabilité cœlomocytes (cytotoxicité) ↘ Activité du microbiote intestinal ↗ Dommages histopathologiques de l'intestin
Ding et al., (2021)	<i>Eisenia fetida</i>	PE ; PLA ; PPC	120	0,125 - 500 g/kg	Sol artificiel	2 – 28	<ul style="list-style-type: none"> ↘ croissance (PE, PPC hautes concentration) ↗ croissance (PLA, PPC 125g/kg) ↗ Mortalité ↘ Reproduction et nb descendants

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Source d'exposition	Durée d'exposition (jours)	Effets
J. Yu et al., (2020)	<i>Eisenia fetida</i>	PE ; PLA	70 - 250	0,5 ; 1 ; 2 ; 5 ; 7 ; 14 % m/m	Sol	28	<ul style="list-style-type: none"> ↗ Neurotoxicité ↘ ou ↗ Stress oxydatif ↘ ou ↗ Défenses antioxydantes Effet d'inhibition-stimulation dose dépendant Changement de composition du microbiote intestinal
Holzinger et al., (2022)	<i>Eisenia fetida</i>	PS (fragment)	125 - 200	0,5 ; 2 % v/v	Sol	56	<ul style="list-style-type: none"> ↘ Reproduction (0,5 %) ↗ CAT (2 %) ↘ MDA (0,5 %)
		PS (fibres)	50 x 1000 - 4000				<ul style="list-style-type: none"> ↘ Reproduction (0,5 %) ↘ MDA (0,5 %)
T. Li et al., (2022)	<i>Eisenia fetida</i>	PS	5 ; 20	100 µg/kg	Sol	35	<ul style="list-style-type: none"> ↗ Dommages histopathologiques de l'intestin ↗ Perméabilité intestinale Translocation microbiote intestinal vers d'autres tissus ↗ Réponse immunitaire ↘ Défenses antioxydantes

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Source d'exposition	Durée d'exposition (jours)	Effets
Xiao et al., (2022)	<i>Eisenia fetida</i>	PS	0,07 ; 1 ; 10	0,5 ; 5 ; 10 % m/m	Nourriture	28	Inhibition de croissance ↗ Stress oxydatif (0,07 µm 10 %) ↘ Réserves énergétiques (0,07 µm 5 et 10 %, 1 µm 5%) Induction dommages histopathologiques
Tang et al., (2023)	<i>Eisenia fetida</i>	PS	25 - 30	0,2 % m/m	Sol	14	Pas de différence de masse ou de survie Pas d'impact sur la diversité du microbiote intestinal ↘ Abondance de certains phylums du microbiote intestinal ↘ Intégrité de la barrière intestinale Perturbation de l'osmorégulation
Jiang et al., (2020)	<i>Eisenia fetida</i>	PS	0,1 ; 1,3	0,1 ; 1 mg/kg	Sol	14	↗ Croissance ↘ Survie (petite taille forte concentration) ↗ survie (0,1 µm ; 0,1 mg/kg) ↗ Lésion histologique intestin ↗ Stress oxydatif ↗ Dommages à l'ADN

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Source d'exposition	Durée d'exposition (jours)	Effets
Schöpfer et al., (2020)	<i>Caenorhabditis elegans</i>	LDPE	57	1 ; 10 ; 100 mg/L	Eau	6	↘ croissance
		PLA/PBAT	40				↘ reproduction
Lei et al., (2018)	<i>Caenorhabditis elegans</i>	PS	0,1; 0,5; 1; 2; 5	1 mg/L	Milieu de culture	3 – Durée de vie	↗ Mortalité ↘ Durée de vie ↘ Taille ↗ Neurotoxicité ↗ Stress oxydatif
Y. Yu et al., (2020)	<i>Caenorhabditis elegans</i>	PS	1	0,1 - 100 µg/L	Eau	3	↘ croissance ↘ reproduction ↗ Stress oxydatif ↗ Perméabilité intestinale
Shang et al., (2020)	<i>Caenorhabditis elegans</i>	PS	1 ; 5	0,01 - 10 % v/v	Eau	3	↘ Durée de vie à (dose dépendance inverse) ↘ Intervalle de défécation
Kim et al., (2020)	<i>Caenorhabditis elegans</i>	PS	0,042; 0,53	10 - 100000 µg/L	Milieu de culture	1	↘ Nb descendants (100mg/L)
				10-100000 mg/kg	LUFA 2.2 ou sol		↘ Nb descendants (530µm 10 et 100mg/kg ; 42µm 100mg/kg)
Fueser et al., (2022)	<i>Caenorhabditis elegans</i>	PS	0,1 - 1	5,5 ; 55 ; 550 ; 687 mg/L	Milieu de culture	4 ; 21 (5,5mg/L)	↗ Réserves lipidiques Tendance à ↘ réserves avec ↗ taille des particules

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Source d'exposition	Durée d'exposition (jours)	Effets
Yu et al., (2023)	<i>Caenorhabditis elegans</i>	PS	1	0,1 ; 1 ; 10 ; 100 µg/L	Milieu de culture	1	<ul style="list-style-type: none"> ↘ Locomotion (1 - 100 µg/L) ↗ Niveaux de glutamate, sérotonine, dopamine et GABA ↗ Neurodégénérescence (expression anormale des gènes codant pour la neurotransmission)
H. Chen et al., (2022)	<i>Caenorhabditis elegans</i>	<hr/> PS (non vieilli) <hr/> PS (vieilli UV)	1	0,1 - 100 µg/L	Milieu de culture	1	<ul style="list-style-type: none"> ↘ Reproduction (100 µg/L) ↘ Reproduction (10 ; 100 µg/L) ↗ Dommages à l'ADN ↗ Apoptose des cellules germinales
De Felice et al., (2021)	<i>Achatina reticulata</i>	PET	<50 - 1000	1 ; 10 % m/m	Nourriture	40	<ul style="list-style-type: none"> ↗ Croissance
Song et al., (2019)	<i>Achatina fulica</i>	PET	1257 x 76,3	14 ; 140 ; 710 mg/kg	Sol	28	<ul style="list-style-type: none"> ↘ Consommation alimentaire ↘ Enzyme antioxydantes ↗ Stress oxydatif ↗ Dommages tissus (estomac & intestin) pour moyenne et forte concentration

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Source d'exposition	Durée d'exposition (jours)	Effets
Song et al., (2020)	<i>Achatina fulica</i>	EPS	N.A.	N.A.	Nourriture (blocs de PS expansé trempé dans du jus de salade)	28	Légers changements de composition de la communauté commensale
(Lahive et al., 2019)	<i>Enchytraeus crypticus</i>	PA6 ; PVC	PA: 13-18; 63-90; 90-150 µm	13-18 & 90-150 µm : 2 - 12 % (m/m)	Sol	21	↘ Reproduction
			PVC : 106-150 µm	63 - 90 µm : 9 % (m/m)			Pas d'effet significatif
Jemec Kokalj et al., (2022)	<i>Enchytraeus crypticus</i>	PP	45 - 55	0,02 ; 0,06 ; 0,17 ; 0,5 ; 1,5 % m/m	Sol LUFA 2.2	21	Pas d'effet sur la reproduction
	<i>Porcellio scaber</i>			0,06 ; 0,5 ; 1,5 % m/m			↘ Viabilité hématocytaire
	<i>Tenebrio molitor</i>						Détection d'une réponse immunitaire
Jemec Kokalj et al., (2018))	<i>Porcellio scaber</i>	PE	137 ; 183	4 g/kg	Nourriture	14	Changements de l'activité énergétique
Kim and An, (2020)	<i>Folsomia candida</i>	PE	2 ; 33 ; 66	10 % m/m	Nourriture	7	Pas d'effet sur la consommation alimentaire ou les réserves énergétiques
							↘ Vitesse de déplacement

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Source d'exposition	Durée d'exposition (jours)	Effets
Zhu et al., (2018)	<i>Folsomia candida</i>	PVC	80 – 250	1g/kg	Sol	56	Altération du microbiome intestinal ↘ Masse animal
Huang et al., (2023)	Arthropodes du sol	LDPE	150	5 ; 10 ; 15 g/m2	Sol	40 ; 130	↘ Reproduction
		PP					↗ abondance (15 g/m2 ; 130 j)
		PLA					↗ abondance (5g/m2 ; 130 j)
		Poly butylène succinate (PBS)					Pas d'effet spécifique
Lin et al., (2020)	Micro arthropodes	LDPE	37	5 ; 10 ; 15 g/m2	Sol (in situ)	287	↘ Abondance microarthropodes (forte concentration)
	Nématodes						↘ Abondance nématodes (exception pour mangeurs de champignons et bactéries)
Selonen et al., (2021)	Enchytrées, collemboles, isopodes, acariens	PES (fibres)	12 x 2870 ; 4000 x 24000	0,02 – 1,5 % (m/m)	Sol ou Nourriture	21 – 28	↘ Survie et reproduction (enchytrées) Pas d'effet significatif (autres)

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Les études portant sur les effets toxiques des MPs sur les vertébrés terrestres comportent moins de modèles biologiques que pour les invertébrés, comme présenté en Tableau 3. Dans ces études, les modèles sont majoritairement la souris (14 études), suivie par le rat et le poulet (4 études chacun), pour finir par les études *in vitro* sur les lignées cellulaires humaines (3 études). Sur les vertébrés terrestres, le polymère le plus largement étudié est le PS (19 études), suivi par le PE (3 études), le PP et le PVC (2 études chacun) et le PET (1 étude). Une étude a également observé les effets de MPs d'origine environnementale sur des souris, mais sans préciser la nature polymérique des MPs impliqués. Les études sur le poulet, le seul vertébré avien du corpus d'études, ont montré que le PS a entraîné chez l'animal exposé par voie orale des perturbations métaboliques, une perturbation du microbiote intestinal, une augmentation du stress oxydatif et de l'inflammation, ainsi que des lésions et nécroses au niveau hépatique, cardiaque et cérébral. La seule étude portant sur la contamination pulmonaire s'est faite chez le rat, et a révélé une diminution de la fréquence respiratoire ainsi que des marques de fibroses pulmonaires après inhalation de PS. Les autres études sur le rat exposé par voie orale rapportent une augmentation du stress oxydatif et une altération des fonctions de reproduction chez l'animal soumis au PS et au PE, mais pas d'effet toxique spécifique du PP. Après contamination par voie orale, le PS a engendré des variations de masse corporelle et une diminution de la masse de plusieurs organes, entraîné une dysbiose du microbiote intestinal, augmenté le stress oxydatif et l'inflammation, engendré des altérations métaboliques, immunitaires et hormonales, provoqué des lésions spléniques, hépatiques, génitales et de la moelle osseuse, et diminué les capacités de reproduction. Le PE, pour le même type d'exposition a entraîné des changements dans le microbiote intestinal, ainsi que le PP (associé à une augmentation de la masse corporelle), quand le PVC et le PET ont entraîné une hyperlipidémie. Les MPs non spécifiés ont quant à eux entraîné un stress oxydatif, une hyperlipidémie, ainsi que des lésions organiques. Les essais sur lignées cellulaires elles, ont montré que le PS augmente le stress oxydatif, l'inflammation, la réponse immunitaire cellulaire, et induit une apoptose ainsi qu'une diminution de la viabilité cellulaire.

Les effets présentés par ces études montrent une tendance générale des MPs à engendrer des lésions et nécroses tissulaires, associées à un état inflammatoire chez les modèles vertébrés, entraînant des défaillances organiques et des problèmes reproductifs. Les types d'effets représentés chez les modèles vertébrés sont semblables à ceux présentés sur modèle invertébré. L'exposition aux MPs entraîne des effets au niveau individuel et sub-individuel. Ainsi les effets sont cohérents entre les différents types de modèles biologiques, appuyant l'hypothèse des effets écotoxiques des MPs sur la faune terrestre. Le MP quasi exclusivement étudié est le PS (19 études sur 24), et la voie digestive représente la quasi-totalité des études. Peu de modèles biologiques sont étudiés, les vertébrés utilisés étant, généralement, utilisés comme proxys pour l'évaluation de l'effet des MPs sur l'être humain. De plus, les études comparatives des effets de différents MPs sur un même modèle sont assez peu nombreuses, quel que soit le type de modèle. Cette lacune concernant les différences d'effets selon la nature polymérique des MPs sera donc adressée durant ce travail de thèse.

IV.2. Microplastiques comme porteurs d'autres contaminants

Les MPs peuvent aussi interagir avec d'autres contaminants environnementaux, tels que les ETMs ou les PO. Ils peuvent également être colonisés par des microorganismes, qui peuvent être pathogènes, facilitant ainsi leur dissémination. Enfin, les MPs peuvent aussi contenir des additifs, dont les effets peuvent eux-mêmes être délétères pour le biote. Les prochaines sous-parties abordent ces différents types de contaminations.

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Tableau 3 : Référencement des études portant sur la toxicité directe des MPs sur les vertébrés terrestres

Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Mode d'exposition	Durée d'exposition (jours)	Effets
Y. Zhang et al., (2022b)	<i>Gallus gallus domesticus</i>	PS	5	1 ; 10 ; 100 mg.L ⁻¹	Alimentation	42	Induction myocardite
							↗ Nécrose cellulaire myocarde
Yin et al., (2023)	<i>Gallus gallus domesticus</i>	PS	5	1 ; 10 ; 100 mg. L ⁻¹	Alimentation	42	↗ Dommages mitochondriaux cardiaques
							↘ croissance cardiomyocytes
							↗ Stress oxydatif cardiaque
							↗ Inflammation cardiaque
							Perturbation métabolisme énergétique
↗ Inflammation intestinale							
Yin et al., (2023)	<i>Gallus gallus domesticus</i>	PS	5	1 ; 10 ; 100 mg. L ⁻¹	Alimentation	42	Destruction de la barrière intestinale
							↗ Nécrose cellulaire intestinale
							Dysbiose du microbiote intestinal
							↗ Inflammation et dommages histopathologiques hépatiques
							Perturbations métaboliques et transcriptomiques
Perturbations du métabolisme lipidique + Stress oxydatif							

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Mode d'exposition	Durée d'exposition (jours)	Effets
Yin et al., (2022)	Gallus gallus domesticus	PS	5	1 ; 10 ; 100 mg.L ⁻¹	Alimentation	42	Perturbations du métabolisme hépatique Lésions de la barrière hémato-encéphalique Présence de lésions cérébrales ↘ Défenses antioxydantes cérébrales ↗ Stress oxydatif au niveau cérébral Induction de nécrose cellulaire au niveau cérébral
Y. Zhang et al., (2022a)	Gallus gallus domesticus	PS	5	1 ; 10 ; 100 mg.L ⁻¹	Alimentation	42	Induction de dysplasie myocardique ↗ Stress du réticulum endoplasmique myocardique Induction d'une autophagie des cellules myocardiques
Lim et al., (2021)	Rat (Sprague-Dawley)	PS	0,145	0,68 ; 1,38 et 2,28.10 ⁵ particule/cm ³	Inhalation	14	↘ fréquence respiratoire ↗ Marqueurs de fibrose pulmonaire

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Mode d'exposition	Durée d'exposition (jours)	Effets
Ilechukwu et al., (2022)	Rat (Wistar)	PS	< 2000	1 ; 5 ; 10 % m/m	Alimentation	90	↘ Volume sperme (10 %) ↘ Motilité spermatique (5 et 10 %) ↘ Spermogramme (quantité) ↘ Testostérone (5 et 10 %) ↗ Altérations histologiques testicule ↗ Stress oxydatif (5 et 10 %)
Ijaz et al., (2022)	Rats (Sprague-Dawley)	PE	N.A.	1,5 mg.kg ⁻¹ .j ⁻¹ masse corporelle	N.A.	56	↗ Stress oxydatif ↘ qualité / quantité spermatozoïdes ↘ LH, FSH et testostérone ↗ Lésions testiculaires
J. Kim et al., (2021)	Rat (Sprague-Dawley)	PP (vieilli chaleur + UV)	85	25 mg.kg ⁻¹ masse corporelle	Gavage	Dose unique (+ 14j surveillance)	Pas de signe de toxicité
				6,25 ; 12,5 ; 25 mg.kg ⁻¹ masse corporelle/j	Gavage	14	Pas de signe de toxicité

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Mode d'exposition	Durée d'exposition (jours)	Effets
Park et al., (2020)	Souris (ICR)	PE	17	3,75 ; 15 ; 60 mg.kg ⁻¹ masse corporelle	Gavage	90	Altération histologique (TD), hématologique et immunologique chez parents
							Altération des mêmes marqueurs et changement sexe/ratio chez progéniture
		PE ; PP ; PS ; PVC ; PET					↗ Inflammation
							Signes de colite
							↗ Stress oxydatif au niveau intestinal
							Dysbiose du microbiote intestinal
Xie et al., (2022)	Souris (Kunming)	PE	150 - 300	4 mg.j ⁻¹	Gavage	7	↗ Diversité du microbiote intestinal
		PP					↗ Masse corporelle
		PVC					↗ Diversité du microbiote intestinal
		PET					↗ Triglycérides
							↗ Masse corporelle
							↗ Triglycérides

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Mode d'exposition	Durée d'exposition (jours)	Effets
X. Chen et al., (2022)	Souris (C57BL/6 mâles)	PVC	2	0,5 mg.j ⁻¹	Gavage	60	<ul style="list-style-type: none"> ↗ Lésions hépatiques ↗ Apoptose hépatique ↗ Stress oxydatif ↘ Expression défense antioxydante Changements dans communauté commensale
Shi et al., (2022)	Souris (C57BL/6)	MP en mélange non spécifié	0,02 - 0,5	1 g.L ⁻¹	Alimentation	180	<ul style="list-style-type: none"> ↗ Stress oxydatif Induction d'une hyperlipidémie ↗ Lésions vasculaires ↗ Altérations histopathologiques hépatiques ↗ Altérations histopathologiques rénales ↗ Altérations histopathologiques cardiaques
Xie et al., (2020)	Souris (balb/c)	PS	5	0,01 - 1 mg.j ⁻¹	Gavage	42	<ul style="list-style-type: none"> ↘ masse corporelle ↗ Stress oxydatif ↘ enzymes antioxydantes dans testicules et ↘ testostérone ↘ spermogramme (quantité/qualité)

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Mode d'exposition	Durée d'exposition (jours)	Effets
Hu et al., (2021)	Souris (balb/c femelle ; C57BL/6 mâle)	PS	10	250 µg/injection (x2 injections)	Injection Intrapéritonéale	11,5	↘ Nb embryons viables ↘ Angiopoïèse placentaire ↗ Altérations immunité materno-fœtale ↗ Marqueurs pro-inflammatoires
Jing et al., (2022)	Souris (C57BL/6J)	PS	0,8 ; 5 ; 10	60 µg.j ⁻¹	Gavage	42	↘ masse rate (5 ; 10 µm) ↗ Dommages histologiques sur moelle osseuse ↗ Dommages histologiques sur rate ↘ Hématopoïèse Changements hématologiques Dysbiose du microbiote intestinal Présence d'altérations métaboliques ↗ Inflammation
J. Wang et al., (2022)	Souris (C57BL/6)	PS	5	5 µg.ml ⁻¹	Alimentation	28	↘ masse splénique ↗ Lésions spléniques Induction suppression immunitaire

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Mode d'exposition	Durée d'exposition (jours)	Effets
Liu et al., (2022)	Souris (CD-1 femelle)	PS	0,8	30 mg.kg ⁻¹ .j ⁻¹	Gavage	35	<ul style="list-style-type: none"> ↗ Accumulation PS dans l'ovaire ↘ Développement des follicules ovariens ↗ Inflammation de l'ovaire ↘ Maturation des oocytes ↗ Stress oxydatif dans les oocytes
Mu et al., (2022)	Souris (ICR mâle)	PS	5	0,1 ; 0,5 ; 1 mg.ml ⁻¹	Inoculation intragastrique	28	<ul style="list-style-type: none"> ↘ Masse hépatique ↗ Lésions histopathologiques hépatiques ↗ saignements et inflammation hépatique, inflammation et transaminases ↗ Stress oxydatif hépatique ↗ Mort nécrose hépatique
Y. Wang et al., (2022)	Souris (C57BL/6)	PS	1 – 10 ; 50 - 100	10 mg.L ⁻¹	Alimentation	30	<ul style="list-style-type: none"> ↗ Lésions histopathologiques vésicales Induction de nécrose vésicale ↗ Stress oxydatif ↗ Marqueurs pro-inflammatoires

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Mode d'exposition	Durée d'exposition (jours)	Effets
							Présence PS dans testicules et ovaires
							↳ Masse corporelle
							↳ Développement des organes génitaux
							↳ Quantité et qualité spermatique
Wei et al., (2022)	Souris (C57BL/6)	PS	5	0,1 mg.j ⁻¹	Gavage	30 ; 44	↗ Lésions testiculaires et ovariennes
							↗ Stress oxydatif au niveau testiculaire et ovarien
							Induction de concentrations anormales en hormones sexuelles
							↳ Fertilité mâle et femelle
							↳ Viabilité embryonnaire
							Pas d'effet sur la croissance
							↳ Masse testiculaire (1mg/j) avec lésions histopathologiques
Wen et al., (2023)	Souris (Kunming)	PS	10	0,01 ; 0,1 ; 1 mg.j ⁻¹	Gavage	35	↗ Malformations spermatiques (0,1 ; 1 mg/j)
							↳ motilité spermatique (1 mg/j)
							↳ Taux de testostérone

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Référence	Modèle biologique	Polymères	Taille de polymère (µm)	Concentrations d'exposition	Mode d'exposition	Durée d'exposition (jours)	Effets
Li et al., (2021)	Souris (CD57 mâle)	PS	5	20 mg.kg ⁻¹ .j ⁻¹	Alimentation	30	↗ MDA ↘ enzymes antioxydante hépatiques
	Lignée foie humain L02			0,5 mg.ml ⁻¹	Via le milieu	0,5	↗ Apoptose ↗ ROS
Dong et al., (2020)	Lignée poumon humain BEAS-2B	PS	1,72	1 - 1000 µg.cm ²	Via le milieu	2	↘ Viabilité cellulaire à 24h (1000 µg/cm ²) et à 48h (10, 100, 1000 µg/cm ²) ↗ Réponse immunitaire et inflammatoire (1000 µg/cm ²) ↗ Perturbation barrière pulmonaire
Wu et al., (2019)	Lignée tumorale humaine (Caco-2)	PS	0,1 ; 5	1 - 200 µg.L ⁻¹	Via le milieu	0,5	Internalisation particules ↗ ROS et dépolariation mitochondries ↗ Dommages sur membranes

IV.2.1. Microplastiques, éléments traces métalliques et polluants organiques

De par leurs émissions concomitantes dans les écosystèmes via les activités anthropogéniques, les MPs ainsi que les ETMs et les PO (comme les produits pharmaceutiques, pesticides et plus largement les phytosanitaires, les hormones, ou un certain nombre d'additifs des plastiques relargués depuis des plastiques, comme décrits dans la prochaine section) peuvent se trouver de façon conjointe dans les environnements terrestres, et il a été montré que les MPs peuvent servir de « cheval de Troie » pour les polluants, dans le sens où ils peuvent adsorber des ETMs et les PO, ou contenir des ETMs et PO qui seront relargués, et ainsi modifier à la fois leur disponibilité environnementale (la fraction d'un contaminant potentiellement disponible pour les organismes) et leur biodisponibilité environnementale (la fraction d'un contaminant environnementalement disponible qu'un organisme absorbe) (Catrouillet et al., 2021; Hildebrandt et al., 2021; International Standardization Organization, 2008; Pauget, 2012). De la disponibilité environnementale dépend donc en partie la biodisponibilité environnementale d'un composé, et *in fine* également la bioaccumulation (l'accumulation totale d'un composé dans les tissus d'un organisme) de ces composés. Une plus grande disponibilité environnementale entraînera donc une plus grande biodisponibilité environnementale, et donc une plus grande bioaccumulation potentielle, dépendamment des capacités d'excrétion de l'animal.

L'adsorption des ETMs par les MPs peut se faire grâce à des interactions électrostatiques directement à la surface des MPs (les régions négativement chargées en surface des MPs interagissant avec les cations métalliques) ou via des complexations de surface (l'oxydation des MPs permettant la production de bandes d'adsorption via la création de composés oxygénés négativement chargés en surface des particules). Les PO peuvent également être facilement adsorbés par les MPs, certains d'entre eux ayant une adsorption facilitée (comme les PCBs ou les HAPs) car étant hydrophobes (Song et al., 2022). Des interactions avec les liaisons hydrogènes ou des interactions π - π (liaisons non covalentes entre cycles aromatiques) peuvent également avoir lieu pour adsorber les ETMs ou les PO après oxydation des MPs, adsorption de biomolécules sur les MPs, ou colonisation biologique de ces derniers. Enfin, une co-précipitation des MPs avec des oxydes ferreux ou des oxydes de manganèse peut également faciliter l'adsorption des ETMs par les MPs (Liu et al., 2024).

Les cinétiques d'adsorption des MPs avec les ETMs ont montré que l'adsorption de ces derniers était rapide. Hildebrandt et al. (2021) par exemple, ont montré une adsorption de 40 à 75 % d'As, Be, Bi, Cr, Fe, In, Pb, Th et Sn après une co-occurrence d'une heure entre ces métaux ($50 \mu\text{g.L}^{-1}$) et des particules de PE et de PET (250 mg) en milieu aqueux. Luo et al. (2020) ont quant-à-eux montré que l'adsorption des MPs sur la matière particulaire du sol était également rapide, avec des cinétiques d'adsorption arrivant à l'équilibre en 3 heures. Cette rapidité dans les cinétiques d'adsorption peut donc potentiellement provenir de la capacité des MPs à interagir rapidement avec d'autres matériaux. Les études portant sur les cinétiques d'adsorption des métaux sur les MPs révèlent que ces cinétiques suivent généralement un modèle de pseudo-second ordre. Ceci indique que l'adsorption des métaux sur les MPs est principalement limitée par un phénomène de chimisorption, phénomène d'adsorption où des liaisons chimiques sont générées entre l'adsorbat et la surface de l'adsorbant (Hildebrandt et al., 2021; Lu et al., 2022; Luo et al., 2020; Qiongjie et al., 2022; Qiu et al., 2022; S. Tang et al., 2023). Cependant, les principaux types de liaisons en cause dans l'adsorption semblent inclure les liaisons hydrogène, les interactions électrostatiques et les liaisons non covalentes (Premarathna et al., 2023), ces liaisons correspondant à de la physisorption, quand l'adsorption n'est pas caractérisée par la création de liaisons chimiques fortes entre l'adsorbat et l'adsorbant. Aussi, les deux types de sorption semblent agir dans ces interactions entre MPs et ETMs, mais la chimisorption est le phénomène limitant l'adsorption, et donc celui qui régule la capacité des MPs à adsorber les ETMs. Les études ont également montré, via l'étude des isothermes d'adsorption des ETMs sur les MPs, que ces phénomènes d'adsorption suivent principalement un isotherme de Langmuir. Ces études montrent donc que l'adsorption des ETMs sur les MPs se fait plutôt via la déposition d'une monocouche d'ETMs à la surface des MPs (Lu et al., 2022; Nguyen et al., 2022; Qiu et al., 2022; S. Tang et al., 2023; Zhu et al., 2022). Les seules exceptions étant pour Qiu et al. (2022) qui ont observé que les isothermes d'adsorption du Hg suivait un modèle de Freundlich sur le PVC, suggérant donc une adsorption multicouche à la surface. Gao et al. (2023) ont trouvé le même résultat en travaillant sur l'adsorption du Hg sur des MPs vieillis, suggérant dans ce cas une adsorption multicouche des ETMs sur la surface hétérogène des MPs quand ceux-ci ont subi un vieillissement.

Il apparaît en effet que le vieillissement des MPs joue un rôle sur leur capacité d'adsorption en augmentant les aspérités à la surface des particules et en modifiant leur morphologie par formation de fissures à leur surface et donc augmentant leur surface spécifique (M. Li et al., 2023; Qiu et al., 2022). Comme vu précédemment, le vieillissement, via des phénomènes d'oxydation de surface, peut également provoquer l'ajout de groupes réactifs à la surface des particules, et donc modifier leur capacité d'adsorption et désorption dans les sols et les compartiments biologiques (Gao et al., 2023; Premarathna et al., 2023; Song et al., 2022). Un autre effet du vieillissement des particules dans les sols peut être leur fragmentation, et donc la diminution en taille des particules, conduisant à l'augmentation de leur surface spécifique, et donc à l'augmentation de leurs capacités d'adsorption en ETMs (Gao et al., 2023; Hao et al., 2023; M. Li et al., 2021b; Song et al., 2022). L'adsorption des PO sur les MPs est régie par les mêmes caractéristiques que celles décrites plus haut pour les ETMs, mais elles sont plus complexes à appréhender, car elles sont également contrôlées par les propriétés intrinsèques des PO, comme leur hydrophobicité, leurs propriétés ioniques et leur structure moléculaire (Chang et al., 2022). Le vieillissement des MPs influence aussi l'adsorption des PO en augmentant la cristallinité des MPs (cf. partie III. Des plastiques aux microplastiques). Or, l'augmentation de leur cristallinité conduit à une diminution relative des zones amorphes, qui ont une meilleure affinité pour les PO, modifiant ainsi leur adsorption et leur désorption. Concernant les cinétiques et les isothermes de sorption des PO sur les MPs, elles suivent plutôt un modèle de pseudo-second ordre, mais selon les PO étudiés, l'adsorption peut être soit via une adsorption monocouche (par exemple pour la norfloxacin) ou multicouche (par exemple pour le carbofuran ou le carbendazime) à la surface des MPs (Song et al., 2022).

En dehors des propriétés des MPs et de leurs co-contaminants qui agissent sur leur sorption sur les MPs, les propriétés physicochimiques des sols sont aussi à prendre en compte. Des facteurs comme le pH du sol, sa structure (via la création de conditions oxydantes ou réductrices) et l'activité microbienne modifiant le pH peuvent changer la charge de surface des MPs, la spéciation des ETMs et la charge des groupes portés par les PO, et ainsi modifier les capacités de sorption des MPs envers leurs co-contaminants. De plus, les proportions de la fraction minérale et organique du sol, ainsi que la présence d'autres métaux essentiels et d'autres biomolécules peuvent, par effet compétitif envers les MPs et/ou les particules du sol également influencer la sorption des ETMs et des PO, et donc moduler leur toxicité conjointe

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avec les MPs sur les organismes du sol (Kim et al., 2023; Luo et al., 2023; Ozturk et al., 2023). Enfin un dernier élément à prendre en compte dans l'adsorption des ETMs et des PO sur les MPs est la présence d'une éco-corona. En effet, une éco-corona est une couche de quelques nm à quelques dizaines de nm se formant à la surface des MPs et constituée d'ions métalliques et inorganiques, ainsi que de biomolécules (Figure 6), provenant à la fois du milieu et de l'activité biologique (bactérienne et fongique) présente dans le sol (Cao et al., 2022; Xingdong Shi et al., 2023). Ces éco-corona peuvent se former en quelques minutes à quelques heures via une sorption de ces ions et biomolécules sur les MPs, changeant ainsi les propriétés de surface (groupes fonctionnels, charges) des MPs, et influençant alors la sorption des ETMs et des PO sur les MPs (Galgani et al., 2020; Yao et al., 2023).

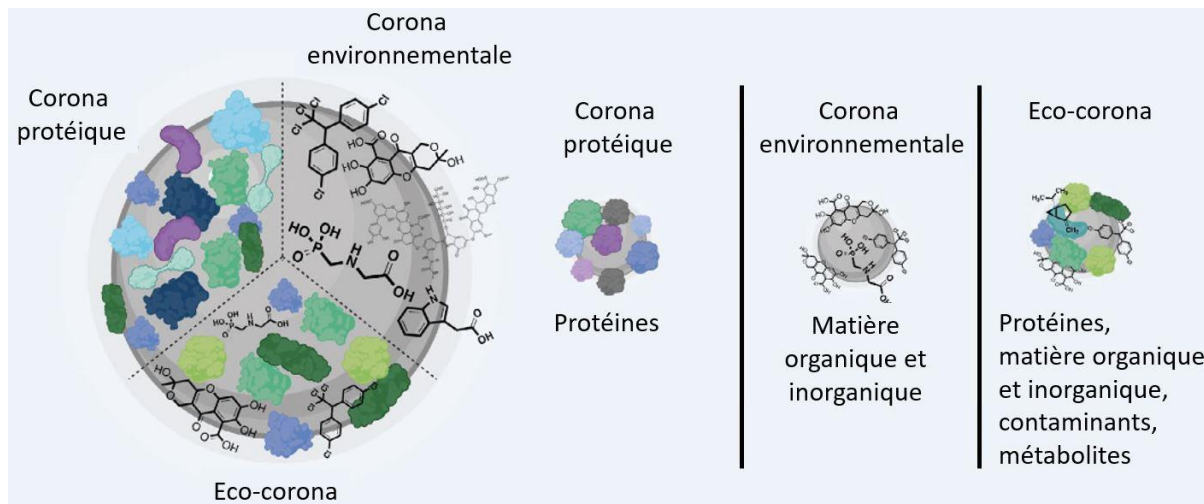


Figure 6: Schéma récapitulatif des différentes molécules organiques et inorganiques pouvant former une éco-corona (adapté de Wheeler et al., 2021)

Ainsi, de nombreux facteurs rentrent en compte dans la modulation de la sorption des ETMs et des PO par les MPs. Ces facteurs sont la nature et les propriétés physicochimiques des MPs et de leurs co-contaminants, les propriétés physicochimiques des sols où ces interactions ont lieu, ainsi que l'activité biologique de ces sols. L'association de ces facteurs fait qu'il peut être difficile de comprendre exactement la mécanistique derrière les changements dans la bioaccumulation des ETMs et des PO dans les organismes concernés par ces co-contaminations. Cependant, la grande majorité des études sur le sujet a montré que les MPs affectent la bioaccumulation de ces co-contaminants en milieu terrestre et peuvent l'augmenter ou la diminuer (Tourinho et al., 2023). Les explications concernant l'augmentation de cette bioaccumulation sont en premier lieu la modulation de leur

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disponibilité environnementale, comme expliqué précédemment. La modification des conditions physico-chimiques dans les organismes après ingestion des MPs chargés en ETMs ou PO peut aussi intervenir. Un relargage de co-contaminant peut avoir lieu après ingestion, via le remplacement de l'éco-corona entourant les MPs dans l'appareil digestif de l'animal par une bio-corona (couche moléculaire similaire à l'éco-corona, composée des biomolécules provenant de l'animal), sous l'action des conditions physicochimiques dans l'appareil digestif (Cao et al., 2022). Le remplacement de l'éco-corona par la bio-corona induisant le relargage des co-contaminants associés à l'éco-corona. En dehors des éco-corona, les MPs peuvent être colonisés par des microorganismes, formant des communautés spécifiques, appelées plastisphères. Dans ces milieux, les microorganismes sécrètent des biomolécules qui peuvent également servir de sites d'adsorption des ETMs et des PO. Ainsi, une fois ingérée, cette plastisphère peut être dégradée dans le tractus digestif de l'animal, relarguant ainsi des co-contaminants. Une autre explication pour la modification de la biodisponibilité de co-contaminants par les MPs peut être un effet physique de ces derniers. En effet, une ingestion de MPs peut potentiellement entraîner des lésions de l'épithélium intestinal chez l'animal, et donc perturber la barrière intestinale. Ainsi, ces lésions pourraient permettre une facilitation du transfert des ETMs et des PO dans les tissus, et donc augmenter la bioaccumulation de ces derniers. A l'inverse, les MPs peuvent également adsorber les co-contaminants via des liaisons fortes (liaisons covalentes avec des fonctions organiques en surface des MPs, co-précipitation avec des ETMs), empêchant potentiellement le relargage des ETMs et des PO depuis les MPs une fois ceux-ci ingérés. Ainsi, les MPs peuvent aussi potentiellement diminuer la biodisponibilité, et donc la bioaccumulation pour le biote des co-contaminants adsorbés à leur surface.

La complexité de ces mécanismes rend l'issue de ces co-contaminations difficile à prévoir en termes d'effets sur le biote. Ainsi, la littérature montre différents effets selon les études, avec parfois des effets contradictoires. Par exemple, Chen et al. (2023) ont montré qu'une exposition au PE (30 μm , 0.02 % m/m dans la nourriture) augmentait l'excrétion urinaire de l'As chez la souris, quand M. Li et al. (2023) ont montré que du PE de plus grande taille (130 μm) ne modifiait pas les concentrations internes en Cd chez *Eisenia fetida* à 0.01 ou 0.1 %, mais augmentait sa bioaccumulation à 1%. Le PE augmentait aussi chez *E.fetida* les concentrations en Cu^{2+} et Ni^{2+} de manière dose dépendante après 21 jours d'exposition (M. Li

et al., 2021). A l'inverse, certaines études ont montré que le PS (0.5 % m/m dans le sol ou les biosolides) n'a pas modifié l'accumulation de Cd chez *E.crypticus* (Ozturk et al., 2023), alors que d'autres ont montré que le PVC diminuait la bioaccumulation d'As chez *Metaphire californica* (Wang et al., 2019). Ces différences de bioaccumulation mènent donc soit à un effet synergique, soit à un effet antagoniste entre les MPs et les ETMs, voire à une interaction sans effet sur le biote. Les mêmes discordances dans les effets ont également été montrées pour les PO chez l'animal. Par exemple le PE (200 – 300 µm, 0.01 % m/m dans le sol) n'a pas entraîné d'effet spécifique sur *C.elegans*, que ce soit seul ou en association avec des molécules à effet antibiotique (amoxicilline), antidépresseur (sertraline), anti-inflammatoire non stéroïdien (Ibuprofène) ou herbicide (simazine). D'autres études à l'inverse ont montré chez l'animal une augmentation de la toxicité des PO. Par exemple, le PE et le PS en combinaison avec des retardateurs de flamme organophosphorés (tris (2 chloroethy) phosphate, ou TCEP et le tris (1,3-dichloro-2-propyl) phosphate, ou TDCPP) chez la souris augmentaient la neurotoxicité, l'induction de stress oxydatif, et perturbaient les métabolismes énergétiques et celui des acides aminés chez les animaux (Xiang et al., 2022).

La contamination conjointe des MPs avec d'autres contaminants, métalliques ou organiques, peut entraîner différents types d'effets, allant du synergisme à l'antagonisme. Ces différences d'effets sont liées à des différences de biodisponibilité elles-mêmes liées aux capacités d'adsorption et de désorption de ces contaminants depuis les MPs. Ces capacités sont elles-mêmes régies par de nombreux facteurs intrinsèques aux MPs et aux autres contaminants, ainsi qu'à des facteurs environnementaux, extrinsèques à ces contaminants. Aussi, il est important d'étudier la capacité des MPs à modifier la disponibilité environnementale et la biodisponibilité d'autres contaminants environnementaux. Bien que des efforts de recherches ont été faits en ce sens ces dernières années en milieu terrestre, les effets diffèrent selon les études, et le nombre de modèles biologiques utilisés reste limité. De plus, les interactions entre les MPs et les ETMs, ainsi que leur résultante en terme de disponibilité, d'accumulation et de toxicité restent encore largement à étudier (Jiang and Li, 2020). De plus, ces études se limitent généralement à un bilan « statique » des concentrations en co-contaminants dans les animaux, ne les considérant pas comme des processus dynamiques, au sens où la toxicocinétique de ces contaminants en présence de

MPs n'est pas étudiée. Ainsi, ce travail de thèse comportera une étude de l'effet des MPs sur le comportement des ETMs dans les sols et l'escargot.

IV.2.2. Microplastiques et additifs

Un autre élément potentiel donnant aux MPs leur toxicité sont les additifs. Comme expliqué en section IV : « Ecotoxicité des microplastiques », les additifs sont des composés chimiques, métalliques ou organiques, qui sont ajoutés de façon intentionnelle aux polymères plastiques pendant leur polymérisation et la fabrication des produits plastiques, pour donner le produit final. Plus de 400 additifs différents sont aujourd'hui clairement répertoriés (bien que leur nombre total soit d'une dizaine de milliers), et ils peuvent avoir différents rôles, comme celui de catalyseur de polymérisation, d'antioxydants, plastifiants, retardateurs de flamme, stabilisants, antimicrobiens, lubrifiants, agents de remplissage, colorants, ou surfactant par exemple (voir Sridharan et al. (2022) pour la liste des principaux additifs associés aux plastiques et leurs applications). Généralement, ces additifs, qui sont ajoutés pour modifier les propriétés des plastiques dans un but commercial, et bien qu'entrant dans la composition du produit fini, ne sont que faiblement liés au polymère, et peuvent donc facilement désorber des MPs dans l'environnement. Leur relargage dans l'environnement, ainsi que leur persistance leur permettent de bioaccumuler dans le biote, et d'exercer des effets toxiques (Ding et al., 2022; Luo et al., 2022; Sridharan et al., 2022). En effet, de nombreux perturbateurs endocriniens sont utilisés en tant qu'additifs dans la production plastique, comme les phtalates, les polybromodiphényléther (PBDE), les bisphénols, le nonylphénol, l'octylphénol, le di(2-ethylhexyl)phtalate (DEHP) ou le butylhydroxytoluène (BHT), ces composés étant souvent également toxiques et carcinogènes (Li et al., 2024), et peuvent donc avoir des effets toxiques sur les organismes du sol. Par exemple (Xu Shi et al., 2023) ont montré qu'une exposition conjointe de DEHP avec des MPs diminuait la récupération après blessure des souris, en augmentant le stress oxydatif et l'inflammation, les effets conjoints d'une co-exposition exacerbant les effets. Ding et al. (2022) ont quant à eux montré que des MPs (PA, PE, PVC, PP et PS), ou leurs additifs extraits (bien que non identifiés) ont diminué la survie et la croissance chez *E.crypticus*, et ont provoqué un changement de leur microbiote intestinal. Ils ont également conclu dans leur étude que les effets des MPs sur les animaux provenaient

majoritairement des additifs que ceux-ci contenaient. D'autres études sur modèles invertébrés aquatiques ont également montré que les additifs relargués des MPs (non vieillis comme vieillis) perturbaient le développement embryonnaire chez *Perma perma*. Une autre étude montrant que des additifs issus de différents plastiques (PP, PET, PS ou PVC) inhibaient la croissance de l'algue *Skeletonema costatum* et entraînaient des effets néfastes sur la survie, le développement, la fertilisation des gamètes et le stress chez *Mytilus galloprovincialis* (Luo et al., 2022).

IV.2.3. Interactions entre MPs et microorganismes

Comme énoncé en partie « IV. Ecotoxicité des microplastiques », les MPs une fois dans l'environnement peuvent être colonisés par des microorganismes, cette colonisation menant à un milieu spécifique appelé plastisphère. Au sein de cette plastisphère, l'association des MPs et des microorganismes qui les colonisent finissent par former un milieu spécifique, avec une communauté bactérienne distincte du milieu environnant (Xingdong Shi et al., 2023). Hormis les possibles changements de disponibilité environnementale de contaminants tiers par ces plastisphères évoqués précédemment, celles-ci impliquent également d'autres risques du fait que les MPs ont été montrés comme pouvant abriter des microorganismes pathogènes. Les MPs possédant une faible densité et étant des particules de faible masse, leur dissémination dans l'environnement par les vents ou la bioturbation peut facilement se faire. Ainsi, les MPs peuvent permettre la dissémination potentielle de microorganismes pathogènes sur de grandes distances (Xingdong Shi et al., 2023; C. Wang et al., 2022; Ya et al., 2022). A ce risque s'ajoute celui lié au transfert horizontal de gènes de résistance aux antibiotiques. En effet, les plastisphères peuvent être considérées comme un milieu privilégié pour ces échanges de gènes de résistance, l'enrichissement bactérien et la proximité des différents microorganismes permettant un potentiel échange de plasmides ou de bactériophages occasionnant des échanges de gènes de résistance. De plus, la présence concomitante de MPs et d'antibiotiques dans les sols, et a fortiori dans les biosolides issus du retraitement des eaux usées, peut aboutir à une sélection dirigée de microorganismes résistants aux antibiotiques, facilitant ainsi de potentiels échanges de gènes de résistance avec d'autres bactéries une fois dans l'environnement (Junaid et al., 2022; C. Wang et al., 2022). Néanmoins, les mécanismes

liés à cet enrichissement et à ces transferts horizontaux sont pour le moment assez peu documentés dans les milieux terrestres, ces problématiques n'ayant été abordées que récemment par la communauté scientifique.

IV.3. Transferts trophiques des microplastiques

Les impacts possibles des MPs sont nombreux, tant de par leur capacité à se propager dans les écosystèmes, que par leur toxicité intrinsèque (qu'elle soit liée à la substance polymérique ou aux additifs adjoints) ou par les contaminants associés aux MPs (vecteurs de contamination d'autres xénobiotiques, risque de propagation de pathogènes). Ces effets toxiques peuvent affecter le biote dans les différents écosystèmes, menaçant donc à terme de perturber la structure des communautés des organismes inféodés au sol, et donc les services écosystémiques rendus (Oliveira et al., 2019; Sridharan et al., 2021). De plus, ces organismes du sol (végétaux comme animaux) peuvent absorber (pour les plantes) ou ingérer, inhaler ou absorber par voie cutanée et tégumentaire (pour les animaux) les MPs. Ainsi, un risque de transfert de ces MPs le long des réseaux trophiques depuis leur base via la prédation est à prendre en compte, des MPs ayant été retrouvés à différents niveaux des réseaux trophiques, tant aquatiques (Nelms et al., 2018; Smith et al., 2018; Zheng et al., 2023) que terrestres (Baho et al., 2021; W. Wang et al., 2022). En dehors de l'étude du contenu digestif d'espèces en milieu naturel, des efforts de recherche ont été faits pour tester la capacité des MPs à remonter les réseaux trophiques via des chaînes alimentaires artificielles en laboratoire (Abdolahpur Monikh et al., 2022; Da Costa Araújo et al., 2020; Da Costa Araújo and Malafaia, 2021; Elizalde-Velázquez et al., 2020; Farrell and Nelson, 2013; Huerta Lwanga et al., 2017). Ces études ont montré un transfert de MPs des proies vers les prédateurs, ainsi que des effets délétères de ces transferts sur les niveaux trophiques supérieurs. Cependant, compte-tenu des difficultés techniques liées à la détection et la quantification des MPs dans les matrices biologiques, ces études de laboratoire ont compensé ces verrous en se concentrant soit sur l'étude du contenu de l'appareil digestif des animaux, soit en effectuant une quantification visuelle des MPs dans les tissus extraits. L'étude de Abdolahpur Monikh et al. (2022) a utilisé une méthode d'analyse indirecte en utilisant des MPs marqués au gadolinium pour estimer les MPs dans les proies (*Hermetia illucens*) entières après dépuration et dans différents

organes du prédateur (*Rutilus rutilus*). D'autres études ont montré un transfert de MPs depuis des plantes jusqu'à des consommateurs animaux, soit en milieu aquatique (Chae et al., 2018), soit en milieu terrestre (Chae and An, 2020; Yanjun Li et al., 2023), avec des escargots du genre achatines comme consommateur final. Dans l'étude de Li et al., (2023), la quantification des MPs dans différents tissus de l'escargot a été réalisée par py-GC-MS, quand Chae and An (2020) se sont intéressés aux effets de ce transfert. Ces études ont montré un transfert de particules des plantes aux escargots, ainsi que des effets des MPs sur le microbiote intestinal et des dommages histopathologiques chez l'animal. Ainsi, bien que le corps scientifique se penche sur les problématiques liées au transfert trophique de MPs dans les écosystèmes, les milieux terrestres sont encore sous-représentés dans les études. De plus, seul un faible nombre d'études procède à une analyse dans les tissus des animaux en excluant l'appareil digestif tout en utilisant des méthodes de quantification n'étant pas basées sur une détection visuelle des particules. Ceci pose un double problème pour l'étude de ces transferts, l'identification visuelle étant sujette à imprécision et un grand nombre d'études se limitant à l'étude des MPs dans le contenu digestif, celui-ci restant un milieu externe, et ne reflétant donc pas la réelle assimilation des MPs par les organismes (Gouin, 2020).

Certaines études ont démontré la présence de MPs dans le tractus digestif d'animaux sauvages, quand d'autres ont montré la capacité effective des MPs à être transférés de proies vers des prédateurs. Cependant, compte-tenu de la difficulté technique liée à la quantification directe de ces MPs dans les matrices biologiques, un grand nombre d'études se base sur une détection directe par visualisation (via utilisation de coloration ou de particules fluorescentes). Cette technique de détection, comme vu dans les chapitres précédents, est sujette à l'erreur dans la quantification des MPs. De plus, peu d'études prennent en compte une réelle internalisation des particules dans les tissus des individus testés, la plupart des travaux portant sur les MPs dans le tractus digestif, ou dans l'individu entier, menant à une surestimation potentielle de l'internalisation réelle des MPs. Ce travail de thèse se penchera donc également sur le transfert de MPs dans les chaînes alimentaires, via une approche visant à quantifier les MPs réellement transférés dans les tissus.

V. *Cantareus aspersus*, indicateur de la qualité des sols

V.1. L'escargot, bioindicateur à l'interface sol-plante-atmosphère

Un certain nombre d'organismes du sol peuvent être utilisés pour évaluer la qualité des sols, tant que les prérequis d'un bon bioindicateur sont respectés (Hodkinson and Jackson, 2005; Hopkin, 1997):

- Être en présence d'un organisme ubiquiste et sédentaire
- Qui participe au fonctionnement de l'écosystème
- Ayant une biologie et une physiologie connue
- Dont l'identification et la manipulation sont aisées
- Dont l'intégration de contaminations (biotiques ou abiotiques) induit des altérations des paramètres biologiques de l'animal permettant d'en tirer des informations sur la qualité du sol
- Qui tolère de fortes contaminations environnementales

Parmi ces bioindicateurs, *Cantareus aspersus* (ou escargot Petit-gris) est utilisé pour évaluer la disponibilité environnementale et la biodisponibilité des contaminants du sol, tant métalliques qu'organiques, ainsi que leurs effets écotoxiques (de Vaufleury, 2015; De Vaufleury et al., 2006; Druart et al., 2017; Gimbert et al., 2008; Gomot-De Vaufleury, 2000; International Standardization Organization, 2018). Ces bioessais d'écotoxicité incluent des tests normalisés d'inhibition de croissance (ISO 15952 : 2018; International Standardization Organization, 2018), ou des tests non normalisés portant sur la toxicité embryonnaire, reproductive, le cycle de vie ou la génotoxicité (de Vaufleury, 2015; Druart et al., 2010, 2017; Louzon et al., 2020; Louzon et al., 2021). Cette espèce, utilisée à la fois pour l'étude de la bioaccumulation et l'écotoxicité des contaminants est maintenant une espèce entrée dans les normes standardisées pour l'étude de la qualité des sols (ISO 24032: 2021; International Standardization Organization, 2021) et a mené au développement d'indices d'intégration *in situ* pour l'évaluation du risque environnemental d'éléments traces métalliques (indice

PARTIE A – L'ESCARGOT CANTAREUS ASPERSUS, INDICATEUR DE LA QUALITE DES SOLS

ERITME) dans les sols (Pauget and De Vaufleury, 2015) ainsi qu'à l'élaboration de valeurs seuils de référence pour ces métaux (Louzon et al., 2020; Louzon et al., 2022).

L'escargot Petit-gris respecte les critères définissant les conditions d'un bon bioindicateur, et son utilisation dans le cadre d'études écotoxicologiques en font un bon bioindicateur de la qualité des sols. De plus, *Cantareus aspersus* vit à l'interface sol-plante-atmosphère, et il peut donc intégrer diverses sources provenant de ces trois voies d'exposition (Figure 7) :

- Digestive, par ingestion de végétaux et de particules de sol contaminés (Scheifler et al., 2006)
- Cutanée, par diffusion de contaminants à travers l'épithélium du pied (Vaufleury and Pihan, 2002)
- Respiratoire, par l'inhalation de gaz et/ou de particules en suspension (Regoli et al., 2006)

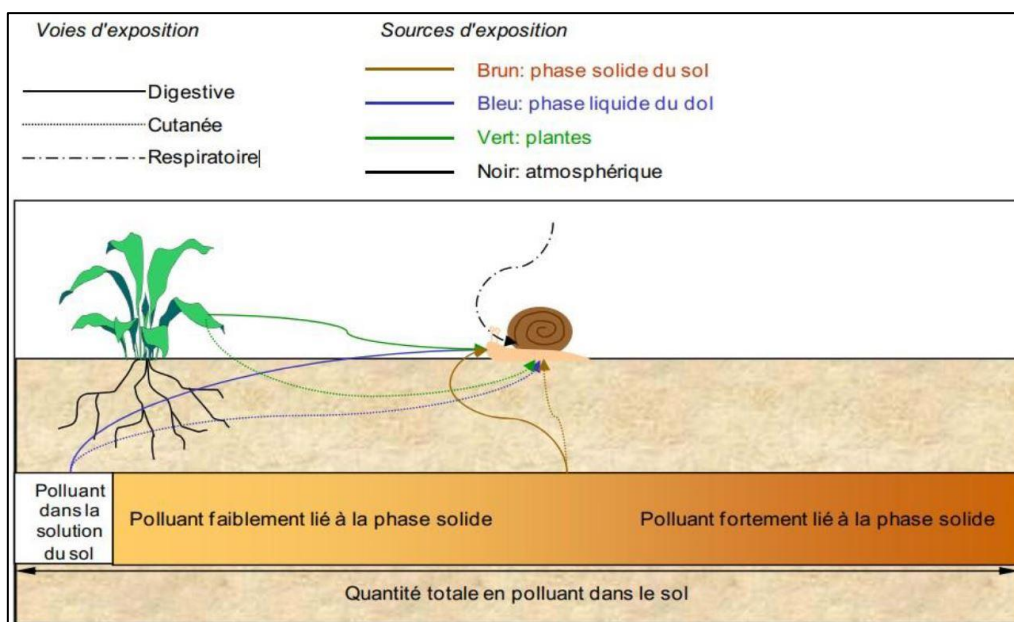


Figure 7: Sources et voies de contamination des contaminants environnementaux pour *Cantareus aspersus* (Scheifler, 2002).

V.2. Éléments de biologie

L'escargot Petit-gris est un escargot hermaphrodite protandre, appartenant à l'ordre des stylomatophore, dans la famille des *Helicidae*. Initialement dénommé *Cochlea hortensis nostra* par Lister et Lister (1685), sa première description a été établie par le naturaliste danois

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Otto Friedrich Müller, le premier nom binomial valide donné par ce dernier étant *Helix aspersa* (Müller, 1774), avant d'être classifié par Jean de Charpentier sous le nom *Cryptomphalus aspersus* (de Charpentier, 1837). Depuis 2015, les systématiciens de l'International Commission on Zoological Nomenclature (ICZN) ont classé l'espèce sous le nom *Cornu aspersum* (International Commission of Zoological Nomenclature, 2015). Cette requalification fait suite à la proposition de Robert Cowie en 2011 de renommer l'espèce (Cowie, 2011), celle-ci présentant des différences morphologiques externes et internes avec les autres espèces du genre *Helix* (Fraussen, 2016). Le nom de genre *Cornu* a été adopté comme nom valide et fait référence à *Cornu copiae* (Born, 1778). Cependant, la classification de l'ICZN fait toujours débat car la description de Born correspond à un individu tératologique de l'espèce *Cornu copiae*, une partie de la communauté des malacologistes considérant donc cette nouvelle classification comme invalide car violant la règle 1.3.2 de l'ICZN prohibant l'utilisation d'individus tératologiques pour la description d'espèce. Bien que l'ICZN ait considéré le nom *Cornu aspersum* valide car Born ne savait pas que l'individu était tératologique, une partie des malacologistes préfère tout de même utiliser le terme *Cantareus aspersus* (Müller, 1774), de par la proximité du Petit-gris avec l'espèce *Cantareus apertus* (Manganelli et al., 2005).

L'escargot Petit-gris présente une coquille d'un diamètre de 30 à 40 mm pour une masse de 6 à 15 g en moyenne. Il possède une coquille dextre de couleur brun-jaune, composée d'une trame protéique externe (la conchyoline, sécrétée par le bord du manteau) à hauteur de 1 à 2 % de la masse coquillière, et d'une partie minérale (carbonate de calcium sous forme de calcite et d'aragonite) pour 98 à 99 % de la masse coquillière. Le corps mou de l'animal est composé de deux parties distinctes : le pied et la masse viscérale (Figure 8). Le pied se compose dans sa partie antérieure de la tête (avec la bouche, deux tentacules oculaires et deux tentacules tactiles, l'orifice génital et le cerveau). Le reste du pied contient principalement du tissu musculaire formant la sole pédieuse impliquée dans le déplacement par reptation, aidée par la sécrétion de mucus, du tissu nerveux, et contient la partie antérieure du tube digestif et les canaux génitaux. La masse viscérale est entourée du manteau, richement vascularisé, qui forme la cavité palléale servant de poumon. La partie antérieure du manteau au contact avec le péristome de la coquille forme le bourrelet palléal. Sur le bord de ce bourrelet palléal s'ouvrent l'anus, l'orifice excréteur et le pneumostome (orifice respiratoire en contact direct avec la cavité palléale et qui agit comme un diaphragme

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pour retenir l'air). Le reste de la masse viscérale, contenu dans la coquille est composé du rein (organe de Bojanus), du cœur, d'une partie du tractus digestif, de la glande digestive (hépatopancréas, qui fait office de foie) et de l'appareil génital hermaphrodite. La bouche de l'animal est suivie par un bulbe buccal entourant la langue recouverte d'une lame chitineuse dentelée (la radula), qui permet de broyer les items alimentaires par va-et-vient. Le bulbe buccal se poursuit par les glandes salivaires, l'œsophage puis l'estomac fusiforme avant de déboucher sur l'intestin, qui forme une double circonvolution autour de l'hépatopancréas. Par la suite d'une torsion à 180° de la masse viscérale de l'escargot pendant le développement embryonnaire, l'intestin forme une boucle dont l'anus est ramené vers la face antérieure. L'hépatopancréas a des rôles entre autres dans le métabolisme, le stockage et la détoxification des xénobiotiques, de sécrétion enzymatique et de réparation de la coquille (Bonnet et al., 2019; Dimitriadis, 2001).

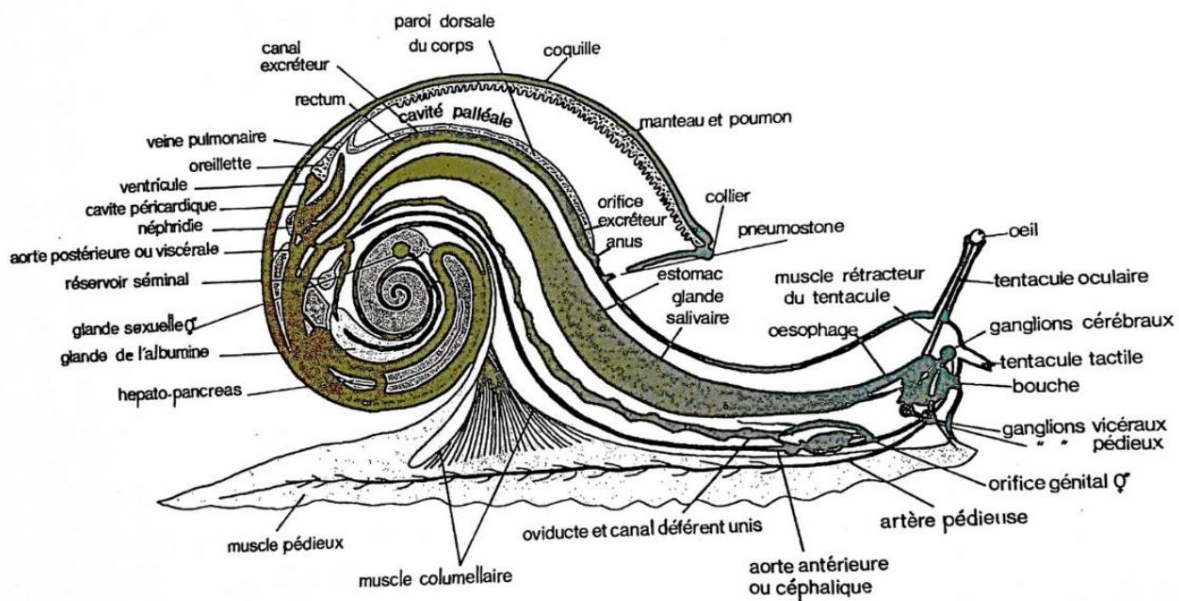


Figure 8: Anatomie de l'escargot *Cantareus aspersus* (Bonnet et al., 2019)

L'escargot Petit-gris est un animal hermaphrodite protandre simultanée, l'appareil reproducteur comportant les appareils génitaux mâles et femelles impliqués dans les fonctions de reproduction, ainsi que l'ovotestis impliqué dans la gamétogénèse (de Vaufléury, 2001). L'acquisition de la maturité sexuelle chez cette espèce se repère via l'apparition d'une bordure péristomiale (Druart et al., 2017), cette étape marquant la fin de la phase de

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croissance des animaux (Gimbert et al., 2008), les individus dans ce cas étant dits bordés. La reproduction est sous le contrôle du système neuroendocrine, le développement du tractus génital femelle, la maturation des oocytes et l'ovulation étant sous contrôle endocrine des corps dorsaux (Griffond and Vincent, 1985) qui sont associés aux tissus des ganglions cérébraux (de Vaufleury, 2001), alors que la différenciation du tractus génital mâle et la spermatogénèse sont sous le contrôle hormonal de cellules appelées cellules du collier, associées au tissu des tentacules oculaires (de Vaufleury, 2001; Flari and Edwards, 2003).

L'escargot Petit-gris est une espèce ubiquiste, originaire d'Europe de l'Ouest et méditerranéenne, elle peut être considérée comme envahissante dans d'autres zones géographiques où l'espèce a été importée, et parfois considérée comme un ravageur de cultures (Kumar, 2020; Potts, 1975). Cet animal est un consommateur primaire herbivore (Iglesias and Castillejo, 1999) avec un comportement géophage pour assurer les apports en calcium et magnésium nécessaires à son développement et à la formation de sa coquille (Gomot et al., 1989).

Le cycle de vie de l'escargot Petit-gris dépend des conditions environnementales, la température, la photopériode, la disponibilité de la ressource alimentaire et l'humidité relative régulant la croissance, la reproduction, et l'activité générale de l'animal. Les variations de températures et d'humidité générant chez le Petit-gris pendant les saisons sèches des périodes d'estivation en période chaude, ou d'hibernation en période froide (Benbellil-Tafoughalt et al., 2009; Iglesias et al., 1996). Dans ces conditions, il se rétracte alors dans la coquille avant de l'obturer avec une sécrétion mucoprotéique renforcée par du carbonate de calcium appelée épiphragme (Bonnet et al., 2019).

L'escargot *Cantareus aspersus* est un invertébré inféodé au sol, qui répond aux critères d'un bioindicateur, et qui peut intégrer différentes sources et voies de contamination. De plus, ce modèle est déjà largement utilisé dans l'étude des effets d'autres types de contaminants (ETMs, contaminants organiques). Il a servi au développement de valeurs guides de références en contaminants, et de plusieurs normes concernant la qualité des sols. Or, l'étude des effets des MPs sur la faune inféodée au sol prend de l'ampleur, mais manque

de diversité dans les modèles biologiques utilisés notamment ceux vivants à l'interface sol-air-plantes. Aussi, cette thèse utilisera l'escargot Petit-gris comme modèle pour l'étude de ces MPs, permettant d'acquérir des données sur un nouveau modèle biologique, et de challenger ce bioindicateur robuste avec l'étude d'un nouveau type de contaminant.

VI. Objectifs

Sur la base des lacunes identifiées dans l'introduction, l'objectif principal de cette thèse est d'aider à la compréhension du potentiel de toxicité des MPs envers les organismes inféodés aux sols, en utilisant l'escargot comme proxy pour étudier la potentielle écotoxicité de ces matières particulaires, leur capacité à interagir avec d'autres contaminants et potentiel à se transférer le long des chaînes alimentaires.

Les objectifs opérationnels sont de déterminer les effets toxiques de différents types de particules de MPs en associant différentes approches chimiques et biologiques sur l'escargot Petit-gris, en tant que nouveau modèle biologique pour l'étude de ces matières particulaires. Plusieurs expérimentations ont été ainsi réalisées *ex situ* pour déterminer les effets toxiques de ces MPs au niveau individuel et sub-individuel (Figure 9).

Plus précisément, trois axes de travail sont visés au cours de ce travail de thèse, dans deux contextes différents, décrits ci-dessous :

Premier volet - Etude de l'écotoxicité de différents MPs à différentes concentrations sur l'escargot *Cantareus aspersus* :

Axe 1) Etudier l'écotoxicité d'un polymère plastique (le polyéthylène basse densité) envers des escargots exposés à différentes tailles de MPs (50 à 500 μm), à différentes concentrations élevées (10 à 50 % v/v) dans la nourriture, sur différents marqueurs individuels et sub-individuels (stress oxydatif, cytotoxicité et génotoxicité) durant une exposition sub-chronique d'individus juvéniles.

Axe 1bis) Etendre nos connaissances précédemment acquises, en étudiant en exposition chronique les impacts de différents polymères (polyéthylène basse densité, polystyrène, polyamide) de plus petite taille (30 à 80 μm), à plus faibles concentrations (0,1 à 10 % m/m) dans la nourriture, sur la mortalité, ainsi que sur les cinétiques de croissance et les cinétiques

PARTIE A – OBJECTIFS

de reproduction (maturité sexuelle, fécondité, fertilité) chez des escargots durant une exposition long terme à l'échelle du cycle de vie de l'animal.

Second volet - Etude des interactions des MPs avec d'autres contaminants et transfert trophique :

Axe 2) Caractériser la capacité des MPs à moduler la disponibilité environnementale et la bioaccumulation de différents ETMs envers l'escargot lors d'une co-occurrence dans les sols, en regardant les effets du PS sur la disponibilité et la biodisponibilité de l'As, du Cd et du Hg à des concentrations environnementales. Pour atteindre cet objectif, les cinétiques de la disponibilité environnementale, et des cinétiques de bioaccumulation des ETMs seront étudiées. Pour la bioaccumulation, l'emphase sera faite sur le côté dynamique du processus chez l'escargot, en utilisant une approche toxicocinétique pour différencier les effets du PS sur la biodisponibilité environnementale et l'excrétion des ETMs chez l'escargot.

Axe 3) Déterminer la capacité de MPs de taille sub-micrométrique à remonter le long de réseaux trophiques depuis une ressource alimentaire contaminée, via un consommateur primaire jusqu'à un prédateur. L'intérêt de cet objectif est double, permettant premièrement de déterminer la capacité des MPs de se disséminer dans l'environnement via les chaînes alimentaires et de contaminer les niveaux trophiques supérieurs depuis des organismes inféodés au sol, et permettant deuxièmement le développement d'une méthode de détection et de quantification, via des méthodes indirectes, des MPs dans les matrices biologiques complexes. Cette partie sera divisée en deux sous parties, la première se concentrant sur la capacité des MPs à être transférés depuis des végétaux cultivés en milieux contaminés vers *Cantareus aspersus*, la seconde portant sur la capacité de ces mêmes MPs à être transférés de *Cantareus aspersus* vers un prédateur, et sur les effets des MPs sur le prédateur en bout de chaîne alimentaire.

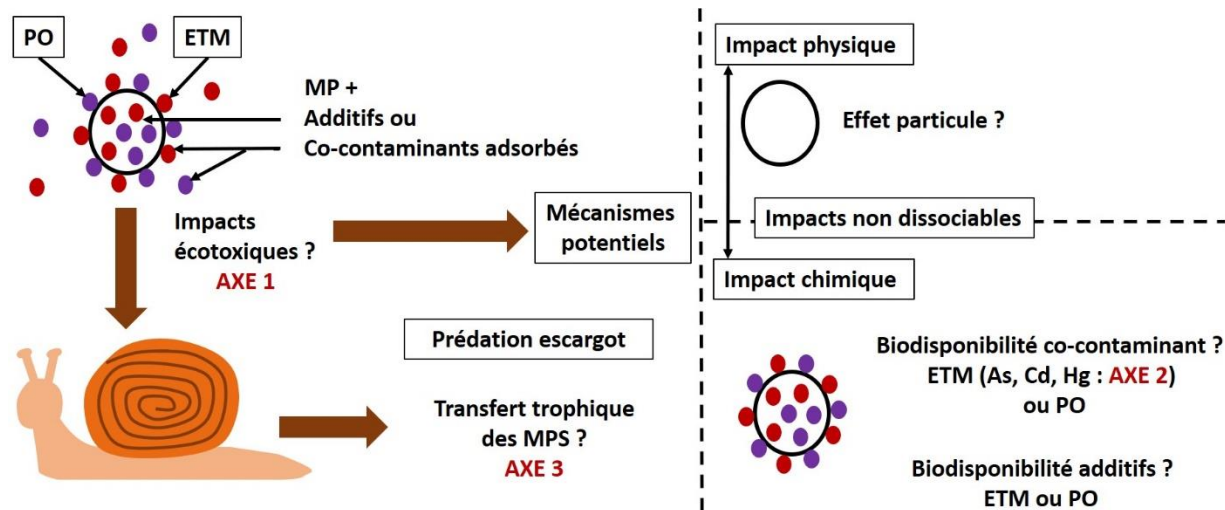


Figure 9: Schéma récapitulatif des objectifs du travail de thèse.

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Partie B – Evaluation ex situ des risques environnementaux des microplastiques

Chapitre I. Evaluation *ex situ* des risques écotoxiques du polyéthylène chez *Cantareus aspersus* lors de bioessai d'écotoxicité en exposition sub-chronique

Titre en anglais : Polyethylene microplastic toxicity to terrestrial snails : size matters

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Résumé

Le plastique est devenu le matériau fabriqué par l'Homme le plus répandu, et les petits fragments de plastique (< 5mm, appelés microplastiques, MPs) s'accumulent dans tous les écosystèmes. Il est maintenant admis que les environnements terrestres représentent un réservoir conséquent pour ces MPs, et ils ne sont devenus que récemment un sujet de recherche, notamment en écotoxicologie. En dépit d'un nombre croissant de preuves montrant les potentiels effets des MPs sur les invertébrés du sol, des efforts supplémentaires sont nécessaires afin de déterminer leurs effets. Le but de notre étude fut de mesurer, à différents niveaux d'organisation biologique, les réponses de l'escargot *Cantareus aspersus* soumis à une contamination en polyéthylène basse densité (LDPE) dans sa nourriture. Des escargots juvéniles ont été exposés à différentes concentrations de LDPE (10, 25 et 50 % v/v) à différentes tailles (tailles médianes de particules (d50) de 120, 292, 340 et 560 µm). Cette étude n'a pas montré de comportement d'évitement alimentaire de la part de l'escargot envers le LDPE. L'ingestion et le processus de digestion dans le tractus digestif des animaux n'a pas mené à une fragmentation quantifiable des particules de MPs. A l'échelle individuelle, les particules de grande taille ont amélioré la croissance des animaux à la plus faible concentration testée, quant à l'échelle moléculaire, seules les particules de petite taille ont provoqué un stress oxydatif, mais sans causer d'effets cytotoxiques ou génotoxiques quantifiables. Les mécanismes sous-jacents restent à élucider, ce qui renforce la nécessité d'améliorer nos connaissances concernant les effets des MPs sur différents modèles biologiques, afin de mieux évaluer leurs risques environnementaux dans les environnements terrestres.

Abstract

Plastic has become the most widespread human-made material and small fragments (<5mm, so called microplastics, MPs) accumulate in all the ecosystems. It is now admitted that the terrestrial environment represents an important sink for MPs and it has only recently become the focus of research, notably in ecotoxicology. In spite of a growing body of evidence regarding the potential effects of MPs on soil biota, more efforts are needed to address issues in this field. The aim of our study was to measure, at different levels of biological organization, the responses of *Cantareus aspersus* snail to low-density polyethylene (LDPE) particles dispersed in their food. Juvenile snails were exposed to a range of LDPE concentrations (10, 25, and 50% v/v) and sizes (median particle size (d50) of 120, 292, 340, and 560 μm). This study showed no snail feeding avoidance toward LDPE. The ingestion and digestion processes along the snail digestive tract did not lead to a measurable fragmentation of the MP particles. At the individual scale, big sized particles improved growth at the lowest exposure concentration tested, whereas at the molecular level, only small sized particles triggered oxidative stress but without causing quantifiable cyto- or genotoxic effects. The underlying mechanisms remain to be elucidated which strengthens the necessity to improve our knowledge on the effects of MPs on various biological models to better evaluate their environmental risks in terrestrial environments.

I.1. Introduction

From the mid-twentieth century until now, our societies have passed from almost totally free from plastics to reliant on them. Plastic materials have become one of the most widespread human-made products in our modern societies. Due to their features (e.g., lightweight and low-cost compounds, high versatility and durability, variety of uses, ...), their production rapidly increased, from 2 million metric tons in 1950 to 359 million metric tons in 2018 (Plastics Europe 2019; Zhang et al. 2020). However, most of these produced polymers are designed for single-use purpose and are thereafter discarded, generating great amount of plastic wastes. For example, it has been estimated that from 1950 until 2015, 6300 million metric tons of plastic wastes have been generated, and 60% of these were discarded or left into the environment (Geyer et al. 2017). Once into the environment, plastic wastes can undergo weathering through abiotic (photooxidation, mechanical abrasion ...) or biotic (microbial activity, ingestion/digestion...) actions modifying their physical or chemical properties. However, plastics are more prone to be fragmented than degraded (Barnes et al. 2009; Song et al. 2017; de Souza Machado et al. 2018a). The fragmentation process of large plastic elements gives more plastic pieces of smaller sizes. These small plastics can be categorized, depending on their sizes, as macroplastics (> 25 mm), mesoplastics (5–25 mm), “large” microplastics (1–5 mm), “small” microplastics (1 μm –1 mm), and nanoplastics (< 1 μm) (Van Cauwenberghe et al. 2015). However, a consensus on the definition of microplastics (MPs), mainly concerning their lower size limit (1 or 20 μm) and upper size limit (1 or 5mm), has yet not been found (Thompson et al. 2009; Frias and Nash 2019). Less visible than large debris, micro- and nanoplastics are nevertheless particularly of concern since their small size gives them special physicochemical properties, facilitating their transport, their retention in environmental compartments, and their transfer in organisms and food chains.

To date, research on MP contaminations and their impacts on biological compartments mainly focused on aquatic ecosystems, and the knowledge on the fate and effects of MPs in terrestrial ecosystems are largely lacking (Akdogan and Guven 2019). However, as a large proportion of plastic wastes is firstly produced, used, and deposited on lands, the terrestrial environment is assumed to be a sink for MPs 4 to 23 times higher than the oceans (Horton et al. 2017). The discrepancy between the body of knowledge concerning MP-related environmental issues in

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aquatic and terrestrial ecosystems can be due to several factors, as (1) the first ever produced work on plastic environmental contamination focused on the Sargasso Sea (Carpenter and Smith 1972), opening the way for the research on marine environment; (2) the effects of macroplastics were rapidly shown on the marine biota, notably concerning the entanglement risk (Gramentz 1988; Laist 1997), driving the attention of the public and (3) ensuring a reliable characterization of MP contamination in soils is challenging, and there is still no suitable method for a full spectrum characterization of the MPs in soils in regard to their diversity in type and size (Wang et al. 2020). Hence, the knowledge on the impacts of the MPs in soils noticeably increased in the last few years, with studies focusing on MP sources (Dris et al. 2015; Steinmetz et al. 2016; Nizzetto et al. 2016; Bläsing and Amelung 2018), their effects on soil physicochemical properties (de Souza Machado et al. 2018b), their biotic transport, and incorporation into the soils (Huerta Lwanga et al. 2017; Rillig et al. 2017). To date, only a relatively limited number of studies have focused on the toxic impacts of microplastics on soil invertebrate micro/meso/macrofauna as recently reviewed by Chae and An (2018), Büks et al. (2020), and Wang et al. (2020). Earthworms, springtails, and nematodes were the main targeted taxa but only two papers report on data concerning terrestrial snails (Song et al. 2019; De Felice et al. 2021). They both concerned polyethylene terephthalate (PET) fibers/particles and the giant snail *Achatina fulica* but they reported contradictory results regarding the induction of oxidative stress after exposure through the diet. According to the diversity of MPs in terms of polymer type, particle size and shape and in regard to the range of inter- and intra-species biological responses, the assessment of their effects on a large panel of organisms is compulsory. Hence, Kim and Rillig (2021) recently estimated, through a species sensitivity distribution (SSD)-like analysis, protective MP concentrations for soil biota and soil properties. They also underlined the lack of available data (notably regarding contrasted soil invertebrate species) as a critical limitation to ensure a broader view of the risks associated with MP contaminations in the terrestrial environment. For such purpose, terrestrial gastropods are relevant candidates regarding their worldwide distribution and their ecological position at the soil–plant–atmosphere interface in various habitats including gardens, woodlands, agricultural areas, and fields (Barker 2001). Among land snails, *Cantareus aspersus* (syn. *Cornu aspersum*; formerly *Helix aspersa*) has long ecotoxicological history and is recognized as a good bioindicator of soil quality (de Vaufleury 2015).

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Therefore, the main objective of our study was to identify and measure the responses of *C. aspersus* snails exposed to a range of sizes and concentrations of low-density polyethylene (LDPE) particles, which is mainly used for food packaging, reusable bags, agricultural films, and is the most widely microplastic retrieved in the environment (Geyer et al. 2017; Scheurer and Bigalke 2018; Plastics Europe 2019). From an operational point of view, we aimed (1) to assess the potential deleterious effects of LDPE at individual (e.g., growth and survival) and molecular (e.g., genotoxicity and oxidative stress) scales and (2) to determine the snail capacities to fragment LDPE particles through their ingestion.

I.2. Material and methods

I.2.1. Biological material and rearing method

The snails used in the experiment (n=360) were juvenile *Cantareus aspersus* (aged 1 month and weighing 1.2 ± 0.13 g) obtained from our laboratory animal cultures. The animals were bred under controlled conditions (temperature of $20 \pm 1^\circ\text{C}$; relative humidity of 80%, photoperiod of 18h; diet = vegetal flour ad libitum) (see details in Gomot-De Vaufleury 2000).

I.2.2. Microplastic

Micronized (< 1mm) LDPE particles were obtained from a plastic products manufacturing company. More precisely, it is a hexane linear LDPE of natural color with a density of 0.93 g.cm^{-3} and containing a UV stabilizer additive suitable for food contact applications. We differentially sieved and sorted this powder into three size classes: 400–500, 200–250, and 50–100 μm , named “big”, “medium,” and “small”, respectively. A fourth size class, corresponding to the initial size mixture (named “mix”), was also studied. The size distribution of each fraction was determined using a laser diffraction particle size analyzer (Beckman Coulter LS230 Laser; Beckman Coulter France S.A.S., Roissy CDG, France). The theoretical and median sizes (d50) are given in Table 4. In each size class, LDPE particles showed a comparable pellet like and angular shape (Fig. S1, in Annex 1).

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Table 4: Table of the theoretical size ranges (μm) and actual median sizes (d_{50} , μm) for the Big, Medium, Small and Mix exposure modalities (in μm).

	Big	Medium	Small	Mix
Theoretical size class	400-500	200-250	50-100	0-1000
d_{50}	560	292	120	340

I.2.3. Experimental design

Following the standard ISO 15952 (2018), the juvenile snails were exposed for 28 days to three concentrations of LDPE particles of each size class incorporated in the food (vegetal flour, Helinove®; Table S1, in Annex 2), i.e., 10, 25, and 50% (v/v). A control was ensured by replacing LDPE particles by 10, 25, or 50% (v/v) lyophilized lettuce. The exposures (15 modalities) were carried out in triplicate in transparent glass containers filled with eight snails (per replicate) and a layer of dampened absorbent paper. The snails were fed ad libitum with uncontaminated or LDPE-loaded food placed in a Petri dish left on the bottom of the container. The day/night cycle photoperiod was 18/6 h, the temperature was $20 \pm 1^\circ\text{C}$, and the air relative humidity was 80–90%. Three times a week, the containers were cleaned, the feces were collected, and the food was renewed.

I.2.4. Measured responses

I.2.4.1. Survival and growth

At each feeding/cleaning session, snail mortality was checked. Moreover, once a week, snail growth was assessed by weighing the fresh mass (whole body) of all the individuals in each container.

I.2.4.2. Oxidative damages

Concentration of malondialdehyde (MDA) in snail digestive gland after 28 days of exposure was used as indicator of oxidative stress (Ohkawa et al. 1979). After sacrifice of two snails per replicate for each exposure modality (with a lethal injection of 100 μL of 1% succinylcholine), 25 mg of digestive gland were homogenized in 250 μL of ice-cold PBS buffer with a FastPrep homogenizer and 1.4 mm beads (MP Biomedicals SARL, Illkirch-Graffenstaden, France). The homogenate solution was then centrifuged at 1600 g for 10 min at 4°C to remove debris. Two

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aliquots (100 μ L) of the supernatant were collected to measure the levels of the total protein content and MDA levels according to the manufacturer's protocols of the BCA Protein and T-BARS Assay Kits, respectively (Cayman Chemical Europe, Tallinn, Estonia). Using a microplate reader MULTISKAN (ThermoFisher Scientific, Illkirch, France), levels of protein and MDA were finally measured at an absorbance peak of 549 nm. MDA results are then expressed relative to the total protein content (nmol/mg protein).

I.2.4.3. Cytotoxicity and genotoxicity

Cytotoxicity and genotoxicity assays were carried out using hemocytes, circulating cells playing a central role in snail innate immunity. These approaches were carried out for each LDPE size but only for snails exposed to the highest MP concentration (i.e., 50% and the related control). At the end of exposure, hemolymph was punctured in one individual per replicate in a 1.5 mL microtube (sterile, DNase free, RNase free, endotoxin free) with a 18 G microlance needle introduced in the snail pericardial area (Louzon et al. 2020). At the cellular level, we assessed cell viability through the hemocyte membrane integrity (HMI) by microscopic enumeration of cells labelled with Trypan blue (Gibco™ Trypan Blue Solution 0.4%, ThermoFisher Scientific) on a Kova slide (Kova™ Glasstic™ Slide 10, ThermoFisher Scientific) according to Strober (2001). A viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue-colored cytoplasm.

At the molecular level, we used the micronucleus (MN) assay, one of the most popular methods to assess genotoxicity of different chemical and physical factors (Sommer et al. 2020). The MN assay was performed according to Louzon et al. (2021). Briefly, immediately after hemolymph sampling, 12 drops of 10 μ L were deposited on a Superfrost slide (ThermoFisher Scientific, Waltham, MA, USA) and kept aside at 80% of relative humidity for 30 min to ensure the adhesion of hemocytes. Then, slides were subjected to a May–Grünwald–Giemsa coloration (Dominique Dutscher SAS, Bernolsheim, France) and covered with Eukitt resin (Sigma Aldrich, St-Quentin Fallavier, France). Finally, the number of hemocytes presenting a micronucleus was counted using an optical microscope (Zeiss Primo Star iLED, Carl Zeiss AG, Oberkochen, Deutschland) and expressed per thousand cells (‰).

I.2.5. Size distribution of LDPE ingested particles

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In order to test whether the LDPE particles are susceptible to fragment through their ingestion and passage in the snail digestive tract, their particle size distributions were determined and compared before (i.e., in the food) and after (i.e., in the feces) their ingestion. The feces were regularly collected over the exposure duration in the 50% LDPE exposed replicates, dried at 40°C, and stored before analyses. Samples of each matrix were digested in 10% KOH during 24h at 60°C under constant agitation. This alkaline digestion procedure was preferred to an acidic one since it allows an efficient removal of organic material while preserving MP particles (Dehaut et al. 2016; Enders et al. 2017). After digestion, the particles were manually collected at the solution surface, rinsed, and analyzed using a laser diffraction particle size analyzer (Beckman Coulter LS230 Laser; Beckman Coulter France S.A.S., Roissy CDG, France) after addition of one drop per sample of a specific deflocculant (Triton X100 (Electrophoresis), Fisher BioReagents™, Illkirch, France).

I.2.6. Statistical analysis

The data on cytotoxicity, genotoxicity, and oxidative stress were analyzed using linear mixed-effect models (lme) with the treatment (LDPE sizes and concentrations) as explanatory factor and the container as random effect (Pinheiro and Bates 2009). Significant differences between treatments were then checked using F-tests (analyses of variance, one-way ANOVA; $p < 0.05$).

Concerning growth, differences in the increase of fresh body mass between treatments were analyzed using generalized additive mixed models (GAMM) (Zuur et al. 2009). The models were fitted using the Gaussian family with identity link on the log-transformed fresh mass data to meet assumptions of normality and homoscedasticity. The variables “particle size,” “LDPE concentration,” and “time” (duration of exposure) were used as fixed explanatory factors, and the container was added as random effect. A smooth term was applied on “time” with the parameter k fixed to 5.

The size distributions of LDPE particles before and after ingestion by snails were compared using generalized additive models (GAM) (Wood 2017). The models were fitted using the Gaussian family with a restricted maximum likelihood method (REML). The variables “ingestion” was used as fixed explanatory factor and a smooth term was applied on “particle diameter” with the parameter k fixed to 11.

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All statistical analyses were performed using R (version 4.0.5) statistical software (packages `gamm4`, `ggplot2`, `mgcv`, `nlme`, `pgirmess`, `r2glmm`, `skimr`, and `visreg`) (R Core Team 2020).

I.3. Results

I.3.1. Survival and growth

No anomalous snail mortality was observed during the 28 days of exposure with an average mortality rate of 4.8% homogeneously distributed in the control and exposed groups (Table S2, in Annex 3).

Regarding growth, the modeled temporal patterns of fresh body mass increase of snails exposed to the different LDPE concentrations and size classes are presented in Fig. 10. When organisms were fed 10% LDPE in their diet, a size effect was evidenced ($p=0.010$). More precisely, for the lowest concentration tested (i.e., 10%), growth was significantly increased for snails exposed to Big particles (+15% at 28 days of exposure compared to control, $p=0.02$) and marginally enhanced for snails exposed to the mix class size (+10% at 28 days of exposure compared to control, $p=0.06$). A comparable trend, although not significant ($p=0.455$), was observed for the exposure to 25% LDPE in food. For the highest LDPE concentration (50%), the growth curves of exposed snails to MP particles are all below the one of the control group. Although the snails exposed to the smallest-sized LDPE particles were 15% smaller than the control individuals (Fig. 10), no significant difference was detected ($p=0.06$).

I.3.2. Oxidative stress

Malondialdehyde (MDA) concentrations measured in the snail digestive gland after 28-days exposure to the different treatments are presented in Fig. 11. The LDPE concentration in food did not influence MDA levels irrespective of the size class ($p=0.820$). However, the size of the particles had a significant effect on MDA concentrations ($p=0.001$). More precisely, the oxidative stress level was in average 2.7-fold higher in the digestive gland of snails exposed to the smallest particle size compared to the control ($p=0.032$).

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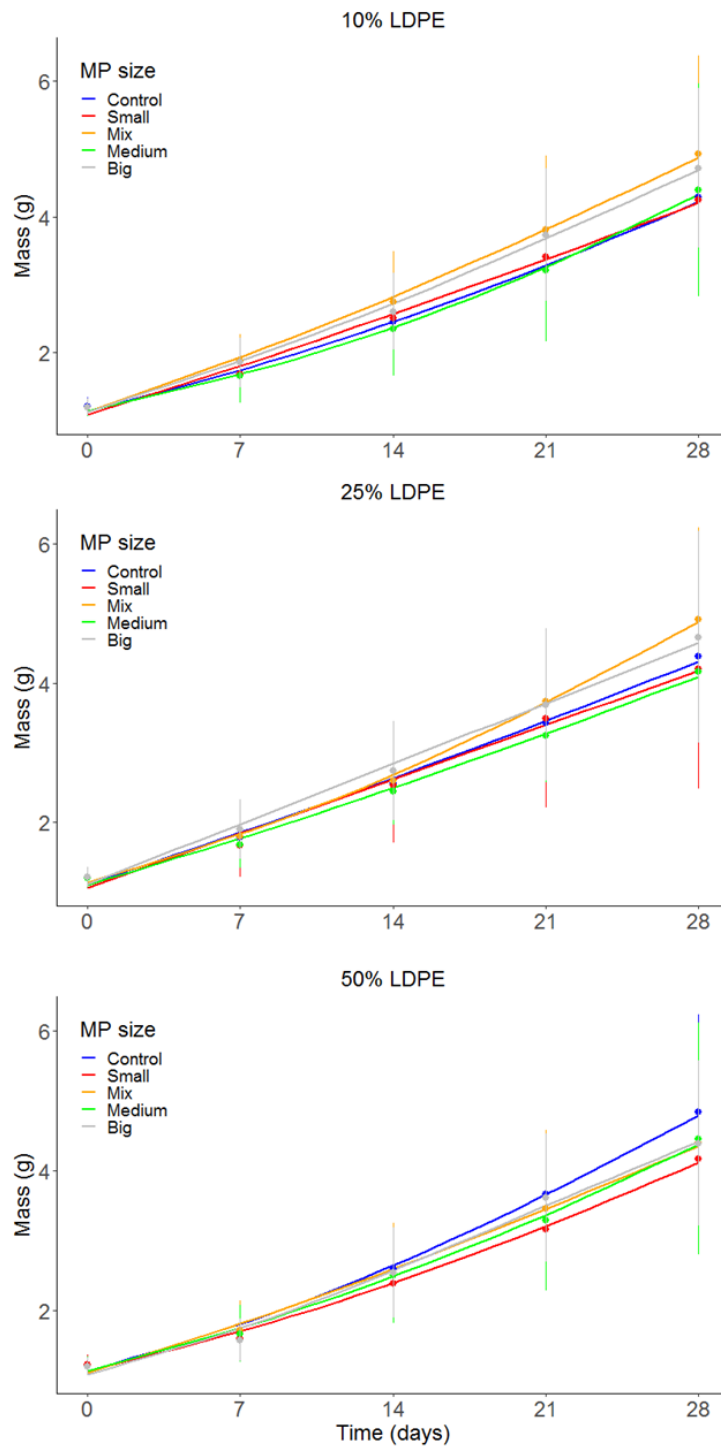


Figure 10: Temporal variation of the snail fresh mass during a 28 days exposure to four LDPE size classes (small, medium, big and mixed sizes) at 10% (top panel), 25% (middle panel) and 50% (bottom panel) LDPE concentrations in food.

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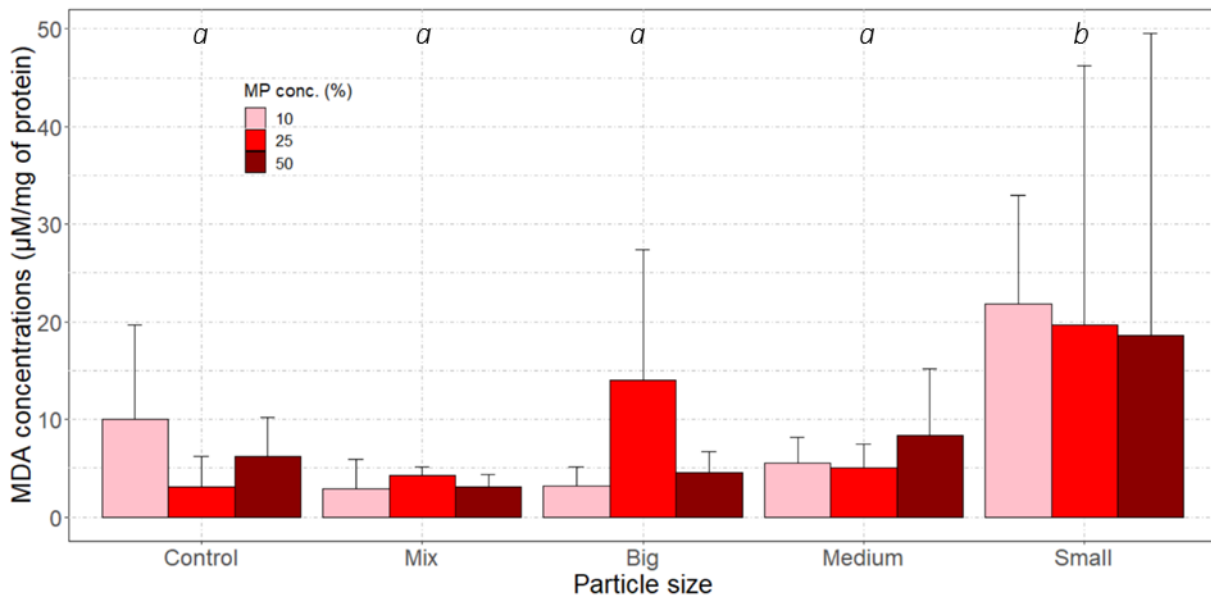


Figure 11: Malondialdehyde (MDA) concentrations (in $\mu\text{g}.\text{mg}^{-1}$ protein) measured in the snail digestive gland after 28 days exposure to the different treatments (LDPE concentrations and size classes). Different letters indicate significant differences ($p < 0.05$)

I.3.3. Cyto- and genotoxicity

At the cellular level, the hemocyte membrane integrity (HMI) was determined in snails after 28-days exposure to the different size classes at 50%LDPE concentration. Data are reported in Fig. 12. Average values ranged between 61 and 89% according to the exposure modality and no significant differences were identified ($p=0.308$).

At the molecular level, the results of the MN assay are presented in Fig. 13. The average number of hemocytes presenting a micronucleus varied between 11‰ in control snails and 47‰ in snails exposed to the medium size class of LDPE particles. However, there was no significant difference between the size classes ($p=0.112$).

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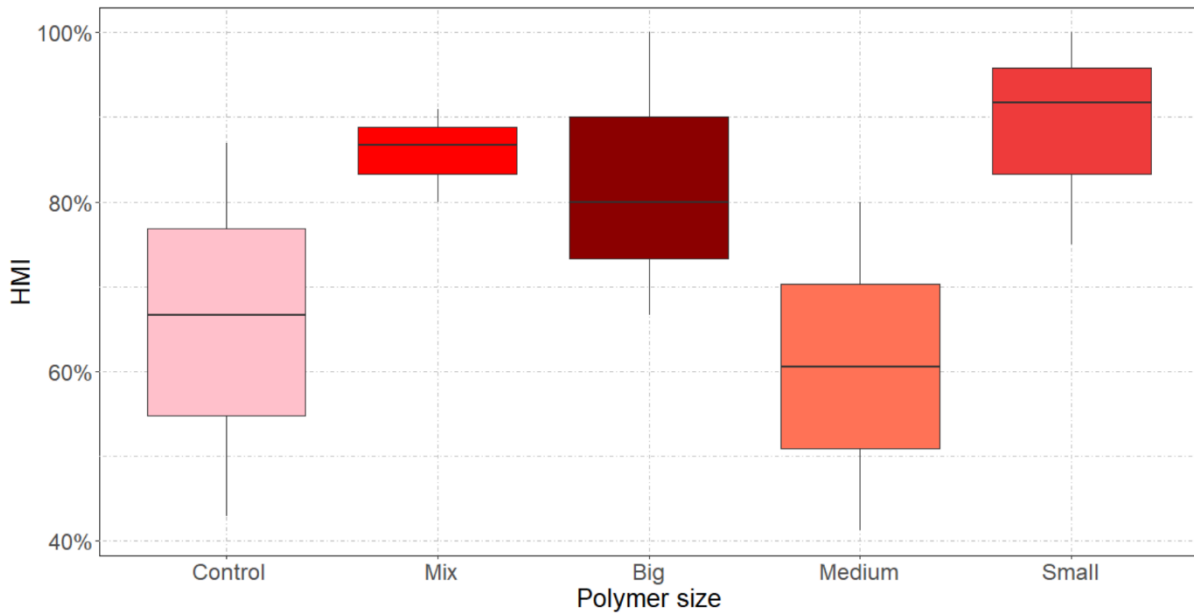


Figure 12: Hemocyte membrane integrity (HMI), determined in snails after 28 days exposure to the different size classes at 50% LDPE concentration

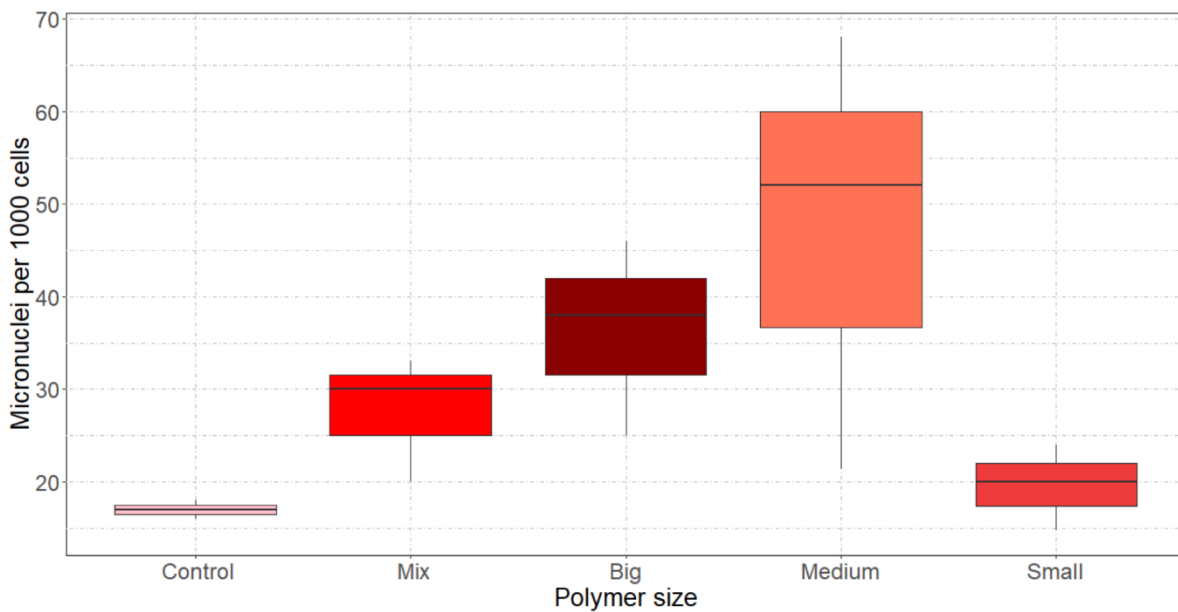


Figure 13: Micronuclei count per 1000 hemocytes in snails after 28 days exposure to the different size classes at 50% LDPE concentration.

I.3.4. LDPE particle fragmentation

The size distributions of LDPE particles were analyzed in the food and the feces of snails exposed to the highest exposure concentration (i.e., 50%). As illustrated in Fig. 14, the size

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distribution before and after ingestion overlapped. Statistical analyses confirmed that the passage through the digestive tract of the snails did not modify the granulometric profile of particles whatever the size class considered ($p= 0.998, 0.802, \text{ and } 0.558$ for big, medium, and small size classes, respectively). For the small size class, the detected large-sized fragments (i.e., around $700 \mu\text{m}$) in both food and feces are attributed to an experimental artifact during the dispersion step.

I.4. Discussion

I.4.1. LDPE particle ingestion and fragmentation by snails

Our study shows that when exposed through their diet, *C. aspersus* snails did not show any avoidance behavior regarding LDPE particles, as no difference in food consumption was observed between the exposure scenarios (data not shown) and particles were retrieved in the feces (Fig. S2, in Annex 4). Size distribution analyses support that *C. aspersus* snails can effectively ingest and egest microplastic fragments measuring at least up to $1000 \mu\text{m}$. However, our data did not allow to demonstrate that this snail specie was able to fragment the ingested LDPE particles. In *Achatina fulica* snails, Song et al. (2019) found notable alterations in the surface morphology of PET fibers extracted from their excrement. Processes of plastic degradation in the environment can be divided into three categories: chemical, physical, and biological (Hakkarainen and Albertsson 2004; Boyle and Örmeci 2020). Such abiotic and biotic mechanisms may also be considered inside the gut of exposed organisms. In helcid snails such as *C. aspersus*, the pH of the digestive tract is close to neutral (Charrier and Brune 2003) and a chemical degradation seems unlikely.

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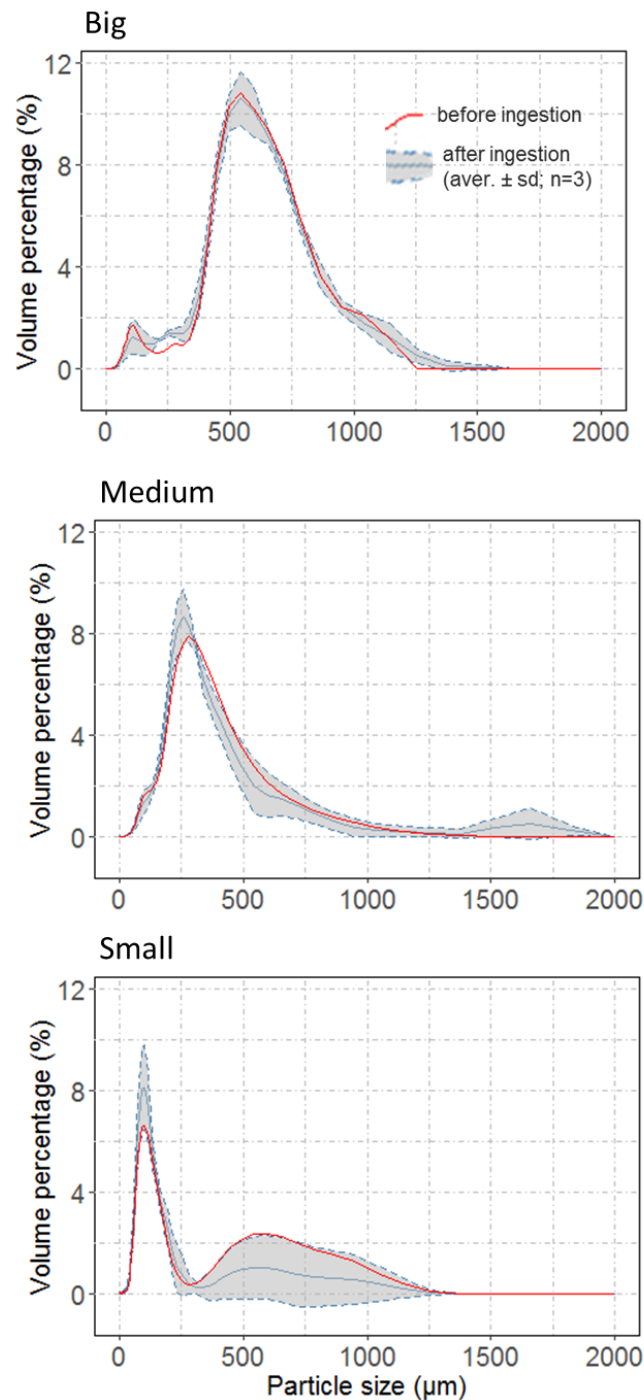


Figure 14: Size distributions of big, medium and small LDPE particles before (red lines) and after (blue lines) ingestion by snails exposed to the concentration of 50% in their diet.

Although snail gut possesses a battery of digestive enzymes able to hydrolyze the ingested plant materials (Charrier and Rouland 2001), their efficiency in chemically altering LDPE particles is probably limited, especially under the anoxic conditions prevailing in the intestinal lumen (Charrier and Brune 2003). Concerning physical alteration of microplastics by

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invertebrates, some studies report the abilities of the freshwater amphipod *Gammarus duebeni* (Mateos-Cárdenas et al. 2020) and the earthworms *Eisenia fetida* or *E. andrei* (Chen et al. 2020; Kwak and An 2021) to fragment PE microparticles after ingestion and digestion. These fragmentation capacities may be related to the morphology of the oral apparatus. In this sense, the arthropod-showing mandibles and the annelid muscular gizzard may be more efficient in comparison to the grazing radula of snails. Moreover, the presence of mineral particles in the diet, such as silica diatoms for gammarids or soil minerals for earthworms, may also facilitate microplastic particle fragmentation in the digestive tract. This impact is probably less marked with the flour provided as food to the snails during our experiment. Finally, the gut bacterial communities may also contribute to the degradation and deterioration of plastic polymer such as PE (Restrepo-Flórez et al. 2014). Hence, the intestinal microbiome of the mealworm *Tenebrio molitor* and the moth *Galleria mellonella* are intricately associated with PE biodegradation in vivo (Brandon et al. 2018; Cassone et al. 2020). It has also been demonstrated a decay of LDPE by bacteria extracted from the gut of the earthworm *Lumbricus terrestris* (Huerta Lwanga et al. 2018). To our knowledge, such capacity of terrestrial snails' microbiota has not been shown yet.

I.4.2. LDPE influence growth but not survival

Our study showed no influence of LDPE particles on the survival of snails, regardless the size or the concentration at which they were exposed. These results are consistent with published studies on another terrestrial snail gender, *Achatina*. After a 28-days soil exposure to PET fibers (0.014, 0.14, 0.71 g kg⁻¹), or a 40-days digestive (food) exposure to PET particles (1 and 10%, w/w), Song et al. (2019) and De Felice et al. (2021) did not highlight any survival impairment in *A. fulica* and *A. reticulata*, respectively. For other soil dwelling invertebrates such as earthworms, PE particles (up to 1000 mg.kg⁻¹) did not induce mortality for *E. andrei* after 2 to 3 weeks of exposure (Rodriguez-Seijo et al. 2017; Kwak and An 2021). A significant mortality was however observed for *L. terrestris* worms exposed to litter containing 28 and 60% PE particles for 60 days (Huerta Lwanga et al. 2016).

Regarding fresh mass, at the lowest exposure concentration (i.e., 10%), a growth enhancement (in comparison to control) was observed for snails exposed to the mix and big size classes, i.e., containing the largest sized LDPE particles. Such coarse particles may have

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facilitated the shredding of food inside gastrointestinal tract of exposed snails and thus promoted the assimilation efficiency and energy intake. This phenomenon was also observed in *A. reticula* snails exposed to similar PET concentrations in food (De Felice et al. 2021). Aside to this mechanical effect, a shift in the gut microbiome driven by the presence of relatively big microplastic particles, as recently demonstrated in PS-exposed *A. fulica* (Song et al. 2020), could also be involved in the improvement of the digestion yield and the weight gain. This growth pattern was however not observed for higher exposure concentrations in food and it was even almost reversed at the exposure of 50% LDPE where the exposed snails tended to grow less than the nonexposed individuals. Such trend meets the definition of a hormetic effect (Jager et al. 2013). At higher exposure doses, the benefits manifested at low dose are exceeded, through modifications in the dynamic energy budget, e.g., in relation with lower nutritional intakes and physiological costs allocated to the management of the stress induced. Further investigations of the snail responses at lower levels of biological organization could bring additional information.

I.4.3. LDPE does not induce cytotoxicity or genotoxicity in *C. aspersus*

Whatever the size of LDPE particles, we did not find significant modifications on *C. aspersus* hemocytes, neither on their concentration in hemolymph (data not shown), nor in their membrane integrity, nor in the induction of micronuclei. Hence, and at least regarding the size ranges tested, LDPE particles did not induce cytotoxicity and genotoxicity in *C. aspersus* snails. Aneugenic and clastogenic damages in hemocytes have been recently demonstrated in *C. aspersus* snails exposed to As, Cd, or Hg contaminated soils using the MN assay (Louzon et al. 2021). This test is therefore relevant as a proxy to determine the chromosomal alteration risks posed by xenobiotics (Hayashi 2016). The cytotoxicity and genotoxicity of MPs are poorly documented regarding soil macroinvertebrates. To our knowledge, only one study carried out the comet assay to assess the genotoxic potential of PS for the earthworm *E. fetida* (Jiang et al. 2020). They observed, in organisms exposed to 0.1 and 1.3 μmPS beads at 100 $\mu\text{g.kg}^{-1}$ and 1 mg.kg^{-1} , DNA fragmentation associated with abnormal shape and nuclei size in intestinal cells. The absence of genotoxic effects in our study may be related to no or very low assimilation of LDPE particles in the snail circulatory system. Only a few studies demonstrated an internalization of microplastics in vivo (i.e., 5 and 20 μm PS beads in mice tissues, Deng et

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al. 2017) or in vitro (i.e., 1–4 μm PE beads in human intestinal epithelial cell lines, Stock et al. 2021). Without more precise data in terrestrial invertebrates, we can only assume that most of the LDPE particles we used were probably not small enough to cross the intestinal epithelium or, if yes, not sufficiently assimilated to exert a direct cytogenotoxic action on snail hemocytes.

I.4.4. LDPE causes oxidative stress in snails

MDA is a product of lipid peroxidation and is therefore a biomarker of the oxidative damage that can lead to alterations of physiological functions, including growth (Smith et al. 2016). Our study revealed that only small-sized particles, for all the concentrations tested, were able to induce an oxidative stress in *C. aspersus* snails.

In the literature, lipid peroxidation and oxidative stress are often involved in the MP toxicity mechanisms through a physical interaction of particles with the gastrointestinal tract wall causing histopathological damages. Increased MDA levels related to lesions of the intestine and stomach villi have been reported in *A. fulica* snails exposed to 1200- μm PET fibers (Song et al. 2019). In earthworms, histological (gut) and molecular alterations were identified in *L. terrestris* exposed to LDPE pellets ranging from 250 to 1000 μm (Rodriguez-Seijo et al. 2017). Using transcriptomic analyses in *Caenorhabditis elegans* exposed to 1- μm PS microbeads, Yu et al. (2020) have also correlated the expression of oxidative stress related genes with intestinal damages. Intestinal damages caused by MP particles appear also more dependent on their size (bigger size leading to damage increase), rather than their composition (Lei et al. 2018). In our study, big particles ($> 300 \mu\text{m}$) facilitated growth (at 10% LDPE) whereas small particles ($d_{50}=120 \mu\text{m}$) induced elevated MDA levels in the digestive gland of snails. The origin of the oxidative stress is then probably not histopathological. Another explanation is the potential internalization of small sized particles at the tissue or cellular levels. Once inside internal compartments, they may then interact with sensitive biomolecules and exert their toxic effect through the induction of an oxidative stress. In our small size class, 2% of LDPE particles measured less than 20 μm , a size compatible with assimilation (Deng et al. 2017; Stock et al. 2021). Although probably insufficient to lead to measurable cytotoxic or genotoxic responses, their internal concentrations may lead to an oxidative stress. Finally, a chemical interaction of MP particles with biochemical reactions through the release, during their

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passage in the gut, of additives present in plastic compounds cannot be excluded. Recently, Kim et al. (2020) evidenced that microplastic toxicity to soil nematodes is highly related to extractable additives. As small particles have a higher specific surface area, their leaching potential of additives may be higher than the bigger ones (Kim et al. 2020). Precise processes involved and mechanistic explanations regarding the induction of oxidative stress following exposure to MPs remain to be elucidated.

I.5. Conclusion

After a 28-days exposure to LDPE particles in food, *C. aspersus* snails showed size dependent responses at different levels of biological organization. At the individual scale, big sized particles improved growth at the lowest exposure concentration tested (i.e., 10%). In the same time, at the molecular level, small sized particles seemingly triggered oxidative stress without causing quantifiable cyto- or genotoxic effects. The underlying mechanisms are still unclear and need further attention. Information is still lacking regarding the potential bioaccumulation of MPs in animal tissues, as well as data allowing to decipher the contribution of the polymer and the associated additives in plastic compounds toxicity. Environmental risk assessment related to MPs would also benefit from carrying out long-term (i.e., life cycle) experiments to investigate potential reproductive and population-based consequences. In this purpose, *C. aspersus* snail appears as a good candidate since we evidenced its relevance as bioindicator of MP toxicity in terrestrial environments.

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Chapitre II. Evaluation ex situ des risques écotoxiques du polyéthylène, du polystyrène et du polyamide sur l'escargot lors d'un bioessai à l'échelle du cycle de vie

Titre en anglais : Ecotoxicological impact of polyethylene, polystyrene and polyamide on the land snail *Cantareus aspersus* on a life cycle experiment

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Résumé

Les plastiques sont le matériel anthropogénique le plus répandu, et de petits fragments de plastique (<5mm, = microplastiques, MPs) s'accumulent dans les écosystèmes. En écotoxicologie, des lacunes existent concernant les effets des MPs sur les organismes du sol. Le but de notre étude a été de mesurer les réponses de l'escargot *Cantareus aspersus* à une exposition au long-court à des particules de polyéthylène (PE), de polyamide (PA) ou de polystyrène (PS). Des escargots juvéniles ont été exposés à une gamme de concentrations (0,1 ; 1 et 10 % m/m) dans la nourriture. De façon générale, une baisse concentration-dépendante de la croissance des escargots a été observée, ainsi qu'une tendance générale à un acquisition précoce de la maturité sexuelle dans les concentrations d'expositions faibles et intermédiaires. Des effets légers (pour le PS) à sévères (pour le PE et le PA) sur la reproduction de l'escargot ont été détectés, mais sans suivre la tendance concentration-dépendante observée pour la croissance. La capacité des escargots à produire des œufs a été principalement inhibée par le PE à 1 % (-32,9 %) et surtout par le PA à 1 % (-52,6 %), ainsi que leur fertilité qui a été réduite de 43,9 et 61,3 %, respectivement. Ces inhibitions ont conduit à une amélioration de la survie, suggérant des changements dans l'allocation énergétique des escargots. Cette étude montre que des tests d'écotoxicité environnementalement pertinents, à l'échelle du cycle de vie, permettent de détecter des effets subtils concernant les réponses individuelles des animaux, démontrant des impacts différentiels selon les polymères considérés.

Abstract

Plastic is the most widespread anthropogenic material, and small plastic fragments (<5mm, = microplastics, MPs) accumulate in the ecosystems. In ecotoxicology, a lack of knowledge exists regarding the effects of MPs on soil organisms. Our study aimed to measure the responses of *Cantareus aspersus* snails to a long-term exposure of Polyethylene (PE), Polyamide (PA) or Polystyrene (PS) particles. Juvenile snails were exposed to a range of MP concentrations (0.1, 1 and 10 % w/w) in food. Overall, a concentration-dependant decrease of the snail growth was observed as well as a general trend toward an earlier maturity acquisition in the low and middle MP exposure concentrations. Mild (for PS) to severe (for PE and PA) effects on the snail reproduction were detected but not following the concentration-dependent trend observed for growth. The snail capacity to produce eggs was mainly affected by PE at 1 % (-32.9 %), and mostly PA at 1 % (-52.6 %), and the fertility was reduced by 43.9 and 61.3 %, respectively. This decrease led to survival enhancement suggesting trade-offs in snail energetic allocation. This study showed that environmentally relevant life-cycle assessment allows to detect subtle effects regarding individual responses, demonstrating differential impacts according to the polymer used.

II.1. Introduction

In half a century of mass production and utilization, plastics have totally impregnated our lifestyles. Thanks to their wide and useful features (*e.g.*, being lightweight, their low-cost production, their diversity in the nature of the polymer used allowing a wide variety of use and their durability, thermic and electric insulation, ...), their production rapidly increased from 2 million metric tons in 1950 to 367 million metric tons in 2020 (Geyer et al., 2017; Plastics Europe, 2020). Their multiple features led plastics to be almost mandatory in our daily lives, from the materials used to build furniture in our homes, to our medical and scientific devices, passing by our daily packaging. For example, PE and PS accounts alone accounts for 44 % of the total plastic production, and are used in various fields including packaging, plastic mulching and industrial uses, while PA accounts for 8 % of the total synthetic fiber production, and is mainly used in the textile industry (Deopura et al., 2008; Letcher, 2020). However, even conceived to last in time or designed for single use purpose, at the end of their using lifetime, plastics are discarded, finally generating plastic wastes, which quantity also increased in time. Geyer et al. (2017) estimated to 6300 million metric tons of plastic wastes were generated by the time of their study, in which 60 % were discarded and could be finally available in the environment. All ecosystems are now contaminated, from remote mountains and plateau in Tibet or Mongolia (Padha et al., 2022), to subalpine lakes in Italia (Della Torre et al., 2023), or in the sediments of the Okavango panhandle (Kelleher et al., 2023). Once entered into the environment, plastic fragments can be structurally or chemically modified through weathering (abiotic or biotic actions modifying their physicochemical properties ; Barnes et al., 2009). These plastic fragments are usually categorized as macroplastics (>25 mm), mesoplastics (5-25 mm), microplastics (5 mm – 1 µm) and nanoplastics (<1 µm), even if these terms have not yet been standardized, and a consensus on their upper (1 or 5 mm) or lower size (1 or 20 µm) have not yet been found (Frias and Nash, 2019; Thompson et al., 2009; Van Cauwenberghe et al., 2015). Several other microplastic (MP) size definitions have been proposed, for example to be any plastic particles with a size less than 5 mm in one dimension (European Chemicals Agency (ECHA), 2019). Microplastics may now be more of concern than macroplastics, since their smaller size give them more reactivity and more size to surface ratio to interact with other environmental contaminants. Their smaller size also allows MPs to be more easily transported, retained in environmental matrices, ingested by the biota and translocated into

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them, thus facilitating their transport along the food chains. The wide range of MPs polymer types, sizes and shapes, associated to the diversity of potentially exposed organisms in ecosystems implies toxicity tests on MPs should be done on a large panel of organisms. Species sensitivity distribution (SSD)-like analysis have already been done, and protective MP concentrations for soil biota have been estimated (Kim and Rillig, 2022). They determined PNEC-like values of 2128 mg.kg⁻¹ for their SSD-like analysis, based on the data available on several plant species, as well as on earthworms, a collembolan specie (*Folsomia candida*) and a nematode (*Caenorhabditis elegans*). However, these authors underlined the lack of data on soil invertebrate species as a limitation of the understanding of the risks posed by MPs in terrestrial environments, terrestrial molluscan notably being absent of such analysis. Furthermore, SSD analysis are based on acute exposure experiments on survival, thus only assessing the effects of the hazardous materials regarding a lone endpoint at the individual scale. Among soil organisms, terrestrial gastropods are relevant candidates for soil risk assessment, due to their ecological position at the soil-plant-atmosphere interface, their ecological roles and their worldwide distribution in a wide range of habitats, like anthropized habitats, woodlands or agricultural lands (Barker, 2001). Among the terrestrial gastropods species, *Cantareus aspersus* has been utilized for years in ecotoxicological studies, and is considered as a good bioindicator of soil quality (de Vaufleury, 2015), and a suitable biological model for the environmental assessment of xenobiotics, such as metals (Gimbert et al., 2016; Louzon et al., 2020), herbicides (Druart et al., 2017) or microplastics (Colpaert et al., 2022). However, to our knowledge, no experiment was done to assess the toxicological effects of multiple polymer types at different concentrations toward gastropods on a full life-cycle bioassay.

To fill the gap concerning the effects of MPs on terrestrial invertebrates, the aim of our study was to assess the long-term effects of a range of concentration of several microplastics on *C. aspersus* snails. In this purpose, land snails were exposed, from juvenile to adult stages, to low-density polyethylene (PE), polyamide (PA) and polystyrene (PS) particles. These MPs are among the most commonly used plastics and thus among the most retrieved MP particles in the environment (Lima et al., 2023; X. Zhang et al., 2022). This long-term life-cycle experiment aims to assess the potential nefarious effects of PE, PA and PS on various endpoints, *i.e.*

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growth, survival and acquisition of the sexual maturity, reproduction, *e.g.*, fecundity, hatching rate, embryonic development time, and fertility.

II.2. Material and methods

II.2.1. Biological material and rearing method


The snails used (n=432) were juvenile *Cantareus aspersus* snails (aged one month and weighing 1.35 ± 0.19 g) obtained from our laboratory animal cultures. The animals were bred under controlled conditions according to Gomot-De Vaufleury (2000).

II.2.2. Microplastics

Micronized pristine PE particles, PA particles and PS spheres were obtained from different plastic-based products manufacturing companies (rotomoulding, 3D printing). The median sizes (d50), and the particle percentages larger than 10 μm and the particle percentages smaller than 100 μm were determined using a laser diffraction particle size analyzer (Beckman Coulter LS230 Laser; Beckman Coulter France S.A.S., Roissy CDG, France). They are given in Table 5.

Table 5: Table of the PA, PE and PS median sizes (d50), standard deviations (in μm), and size distributions (in %) over 10 μm and under 100 μm .

	PA	PE	PS
Median size	57.8	31.24	80.09
sd	2.83	31.24	1.07
% > 10 μm	94.4	93.3	100
% < 100 μm	93.9	96.5	100



II.2.3. Experimental design

Based on the standard ISO 15952 (International Standardization Organization, 2018), the snails were exposed for 112 days (= 4 months) to three concentrations (0.1 %, 1 % or 10 %, w/w) of PE, PA or PS particles incorporated in the food (vegetal flour, Marion®; SAS aliment Marion, 17 impasse du Champs Gaillard, 01290 Saint Jean sur Veyle). An uncontaminated lettuce control (L) was ensured by replacing the MP particles by 0.1 %, 1 % or 10 % (v/v) lyophilized lettuce. The exposures (12 modalities) were carried out in triplicate in transparent glass containers containing a dampened absorbent paper, with 12 snails per replicate. The snails were fed *ad libitum* with uncontaminated or with PA-, PE- or PS-contaminated food placed on glass Petri dishes left in the bottom of the glass containers. The snails were reared under controlled conditions with an 18/6h day/night photoperiod, a room temperature set as $20 \pm 0.1^{\circ}\text{C}$ and an air relative humidity at 80 %. Three times a week, the containers were cleaned and the food was renewed.

II.2.4. Measured responses

II.2.4.1. Survival and growth

At each feeding session, the snail mortality was checked in every container. The snail growth was assessed once a week by weighing the fresh whole-body mass of each individual.

II.2.4.2. Sexual maturity

Sexual maturity was assessed at each feeding session with an examination of the shell. The animals expressing a curvature on the shell edge were considered mature, as this curvature can be used as an indicator of the sexual maturity in snails (Louzon et al., 2020; Lucarz and Gomot, 1985).

II.2.4.3. Fecundity and fertility

When first matings were observed, glass nesting boxes filled with clean damp horticultural compost were disposed in each box, as described in Druart et al. (2010). Each day, the nesting boxes were checked for clutches, and each clutch was retrieved, rinsed with tap water, counted and placed for incubation in glass Petri dishes on dampened filter paper (Druart et

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al., 2010). At the end of the embryonic development, the number of hatchlings for each clutch was determined.

Fecundity was determined as the average number of eggs laid per sexually mature animal per replicate, and the fertility was determined as the average hatchling number per sexually mature animal per replicate. The embryonic development time (*e.g.*, the time between the eggs laying and the eggs hatching), and the hatching rate (*e.g.*, the percentage of laid eggs that hatched) were also determined.

II.2.5. Statistical analyses

The data on mortality, embryonic development and hatching rates were analyzed using linear mixed-effects models (lme) with the modalities (Lettuce, PA, PE or PS, as well as concentrations) as explanatory factor and the container as random effect at 28, 56, 84 and 112 days, corresponding to 1st, 2nd, 3rd and 4th month of exposure. These dates correspond to the growth period, maturity acquisition, reproduction and end of experiment periods. Significant differences between modalities were then checked using F-tests (analyses of variance, one-way ANOVA; $p < 0.05$), followed by Tukey post-hoc test for multiple comparisons.

Growth parameters were modeled using a Gompertz model (Gimbert et al., 2008), using the equation 1:

$$W_t = W_{max} \times e^{(-e(-k_g)x(t-I))}$$

With W_t the snail mass at time t (in g), W_{max} the maximal mass reached by the snails (in g), k_g the growth constant (in d^{-1}), t the time (in d) and I the time at the inflection point of the growth curve (in day).

Sexual maturity, fecundity and fertility parameters were modeled using a logistic model, using the equation 2:

$$Maturity | Fecundity | Fertility = \frac{A}{1 + e^{-k(t-I)}}$$

With “Maturity | Fecundity | Fertility” the endpoint considered (in % of snail sexually mature, in number of laid eggs per mature snail or in number of hatched eggs per mature snail, respectively) at a time t (in d), A the maxima of the considered endpoint, k the associated

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constant (d^{-1}) and I the time at the inflexion point of the curve (in d) corresponding to the peak of the considered endpoint (peak of maturity acquisition, peak of egg laying or peak of hatching).

Growth, maturity, fecundity and fertility models were fitted using a non-linear mixed effect procedure (nlme) integrating the exposure modality (Lettuce, PA, PE or PS, as well as concentrations) as fixed effect and the container as random effect. Significant differences between modalities were then checked using F-tests (analyses of variance, one-way ANOVA, $p < 0.05$), followed by Wald tests for post-hoc multiple comparison.

To assess if the individual effects observed in the animals were potentially partially linked to trade-offs in the energy allocation budget, the relationships between survival, growth and investment in reproduction were investigated using generalized linear mixed effect models (GLMMs). Quasi-binomial logistic regressions were used due to overdispersion. In the first two models, survival was categorized as the response variable (in term of mortality ratio), while the cumulated laid egg number or the mass were categorized as the explanatory variables for the first and the second models, respectively. In the third model, growth was categorized as the response variable and the cumulated egg number was categorized as the explanatory variable. In the last model, the cumulated egg number was the response variable, and the growth was used as the explanatory variable. A first step of selection using a full model (with the response variable modeled as the response of the explanatory variables, and integrating the exposure modalities, the concentrations, as well as their first order and second order interactions) was done, and the different models were simplified by removing the non-significant interactions one by one. The modeling process was done iteratively until the only explanatory variables kept in the final models were the significant explanatory variables and their significant interactions. The exposure modality (Lettuce, PA, PE or PS, as well as concentrations) and their significant interactions were integrated as fixed effect in the models, and the container as random effect. Significant differences between modalities were checked using type II Chi-squared tests, followed by Wald tests for post-hoc multiple comparison.

Data analyses were done using RStudio V.2022.12.0 build 353 (R Core Team, 2022), and using the dplyr V.1.1.0 package (Wickham et al., 2023) as well as the nlme V3.1-160 package (Pinheiro et al., 2023). Energetic trade-offs analyses were done using the package MASS V7.3-

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60 (Venables and Ripley, 2002) as well the car package V3.1-1 ((Fox and Weisberg, 2019). The graphics were done using the ggplot2 package V3.4.0 (Wickham, 2016). Data analyses were done using RStudio V.2022.12.0 build 353 (R Core Team, 2022), and using the dplyr V.1.1.0 package (Wickham et al., 2023) as well as the nlme V3.1-160 package (Pinheiro et al., 2023). Energetic trade-offs analyses were done using the package MASS V7.3-60 (Venables and Ripley, 2002) as well the car package V3.1-1 ((Fox and Weisberg, 2019). The graphics were done using the ggplot2 package V3.4.0 (Wickham, 2016).

II.3. Results

II.3.1. Growth

The growth modeling showed different response-type regarding the polymer type and the concentration (Figure 15). A summary of the growth modeled parameters for each polymer and each concentration, is provided in supplementary material (Table A.1., in Annex 5). Concerning the 0.1 % concentration exposure, the maximal snail mass (W_{\max}) was lower in the L modality vs the contaminated ones ($p < 0.01$), with a W_{\max} of 5.17 ± 0.13 g for L and a 12.45 %, 13.09 % and 8.29 % weight increase for the PE, PA and PS modalities (Table A.1., Annex 5). The pattern changed for the 1 % exposure concentration, with lowered W_{\max} for PE and PA (-7.14 % and -11.09 %, $p = 0.02$ and $p < 0.01$, respectively) vs L (5.73 ± 0.13 g), and no difference was observed between L modality and the PS one. Regarding the 10 % exposure, PE modality has the lowest W_{\max} with a 11.23 % mass decrease vs L, and was different from the other modalities ($p < 0.01$). At 10 % exposure, the growth constant (k_g) was also different from L for PA and PS (+32.26 % and +30.73 %, $p = 0.03$ and 0.04, respectively), and PE had no difference from L or the other modalities. No difference was observed for k_g at 0.1 or 1 % (Table A.1., Annex 5). Finally, the time at the inflexion point of the curve, *i.e.*, the date of the growth peak (I), was not influenced by the exposure to MP particles, whatever the polymer.

Concentration effects for each polymer have also been investigated. The control group showed an increase of W_{\max} with the lettuce concentration, with increased W_{\max} at 1 % and 10 % lettuce concentration vs 0.1 % ($p < 0.01$). For 1 % and 10 % PE exposed snails, a decrease of both the final mass (W_{\max} ; $p < 0.01$) and the time to reach the growth peak (I, $p = 0.03$) were observed compared to 0.1 % MP. The exposure to PA led to a U-shaped response curve

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for W_{max} , with a lowered mass in 1% PA concentration vs 0.1% ($p < 0.01$) and vs 10% ($p < 0.01$). Finally, the PS modality showed an inverse-U-shaped-like response with a higher W_{max} at 1% PS concentration ($5.99 \pm 0.12g$) vs 0.1% (-6.5%; $p = 0.02$) and 10% PS (-4%; $p = 0.1$), though no difference were found between the two modalities.

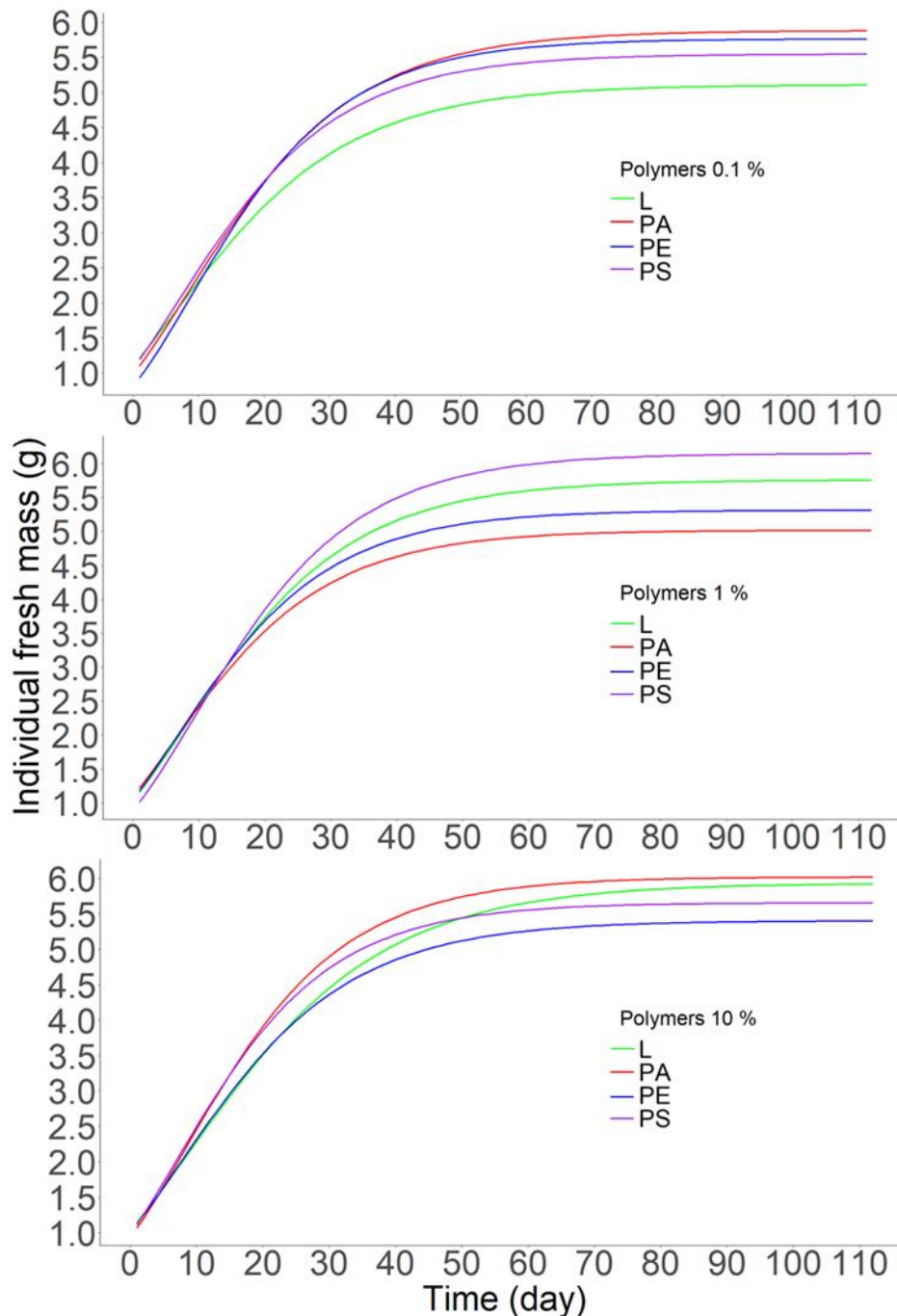


Figure 15: Snail modeled mass evolution curves (in g) at 0.1% (upper panel), 1% (middle panel) or 10% (lower panel) MP or lettuce control groups, during the experiment (time in days).

II.3.2. Sexual maturity parameters

The modeled sexual maturity acquisition curves are shown in Figure 16 and the values of the estimated parameters are provided in supplementary material (Table A.2., in Annex 6). Overall, after 4 months exposure the percentage of mature individual (A) did not differ, regardless the polymer or the concentration. However, the maturity acquisition dynamics were significantly modified by the exposure to MPs. At 0.1 % concentration, the peak of sexual maturity occurred earlier in the exposed modalities (-9, -7, and -9 days d for PE, PA and PS, respectively) vs in L (at 46.25 ± 1.63 d; Table A.2., Annex 6). At 1 %, the peak of maturity occurred earlier in PE (- 8 days) and PS (-7 days) vs L (46.05 ± 3.09 d, $p = 0.02$) and vs PA (45.88 ± 1.58 d, $p < 0.01$), while it was lowered in PS only vs PA ($p < 0.01$). Concerning the highest exposure concentration (10 %), the peak of maturity acquisition was later in PE than in PS ($p = 0.037$).

Regarding the concentration effects for each polymer, in the L modality, the sexual maturity constant (k) was higher at 1% concentration than at 0.1% ($p = 0.02$) and the maturity peak occurred sooner at 10 % than at 0.1 % ($p = 0.02$). In the PE modality, the percentage of maturity was lowered at 1% and 10 % vs 0.1 % ($p=0.02$ and 0.03 , respectively), and the maturity peak (I) was significantly delayed at 10 % (+4.5 days). For the PA modality, the snail sexual maturity was delayed at 1 % vs the other exposure concentrations. Finally, the PS modality showed a decreased maximum mature snail percentage at 1 % vs 0.1 % ($p = 0.02$).

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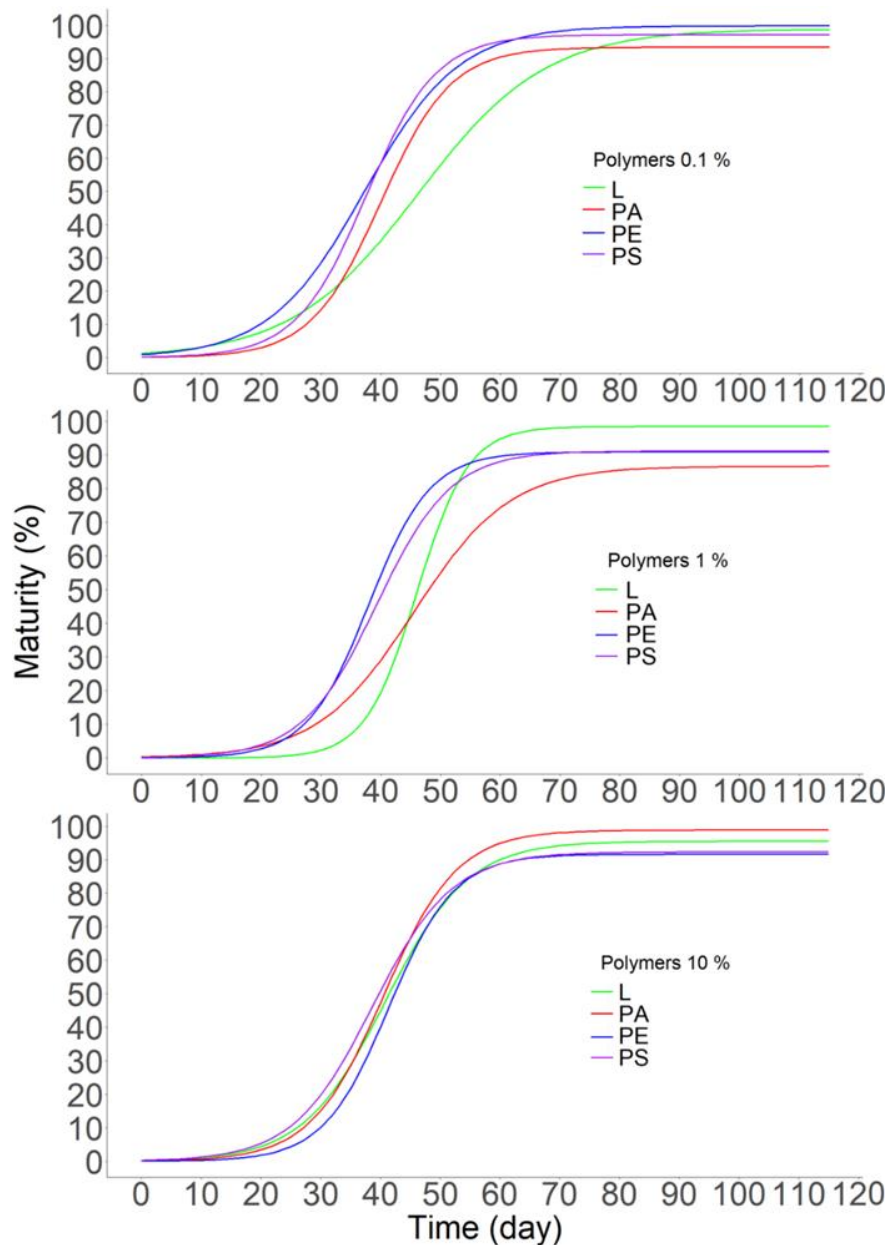


Figure 16: Snail modeled sexual maturity acquisition (in %) at 0.1 % (upper panel), 1 % (middle panel) or 10 % (lower panel) MP or lettuce control groups, during the experiment (time in days).

II.3.3. Fecundity parameters

The modeled fecundity curves for the control and the treated groups are given in supplementary material (Figure A.1., Annex 7) and the values of the estimated parameters are provided in Table 6. The fecundity patterns of the exposed snails varied amongst the modalities. Regarding the MP type, at 0.1 % exposure concentration, the only significant difference was observed in the peak of egg laying (I) in the PA group (71.81 ± 2.99 d) vs the PE group (83.32 ± 4.88 d). At 1 %, more eggs were laid in L (143.68 ± 13.66 eggs) and PS

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(132.43±7.33 eggs) vs PE (-32.90 %, $p < 0.01$) or PA (-52.59 %, $p < 0.01$) exposed snails. The egg laying peak (I) appeared earlier in the MP exposed animals (-10, -11 and -20 days for PE, PS and PA, respectively) vs in L. At the highest exposure concentration (10 %), less eggs were laid for PE (-14.49 %, $p = 0.04$) and PS (-14.51 %, $p < 0.01$) vs L; the egg number was even lower for PA exposure (-46.41 %) and different from L, PE and PS ($p < 0.01$).

Regarding the concentration effect, in the control, the snails fed with 0.1 % lettuce laid less eggs compared to those exposed at 1 % ($p < 0.01$) and 10 % ($p = 0.02$). For PE, the exposure to 10 % led to a higher number of eggs per snail compared to 0.1 % ($p = 0.046$) or 1 % ($p = 0.01$). The exposure to PA showed an inverse trend, with snails exposed to 0.1 % laying more eggs compared to other exposure concentrations ($p=0.02$). Regarding PS, an exposure to 10 % lowered the number of eggs laid compared to 1 % ($p < 0.01$) and the egg laying peak was reached earlier at 1 % ($p = 0.02$) and 10 % ($p < 0.01$) vs 0.1 %.

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Table 6: Modeled fecundity and fertility parameters: A (max number of laid eggs or hatched eggs per snail), k (the associated constant, in d-1) and I (the time at the inflexion of the curve, in d) for control and exposed modalities at each concentration, with the associated percentage of variation for each MP modality compared to the control in each concentration. The lowercase letters represent the significant differences for each parameter between modalities for the same concentration. The uppercase letters represent the significant differences for each parameter between concentrations for the same modality.

[MP] Polymer	Fecundity parameters				Fertility parameters			
	0.10%				0.10%			
	L	PE	PA	PS	L	PE	PA	PS
A	100.05 ± 14.63	99.37 ± 13.52	104.14 ± 7.64	124.28 ± 32.19	58.09 ± 8.55	67.95 ± 8.04	67.39 ± 5.37	91.60 ± 24.69
% variation	- a / A	-0.68 a / A	4.09 a / A	24.22 a / AB	- a / A	16.97 a / A	16.01 a / A	57.69 a / AB
k	0.09 ± 0.03	0.1 ± 0.03	0.12 ± 0.04	0.07 ± 0.02	0.08 ± 0.03	0.1 ± 0.03	0.1 ± 0.03	0.06 ± 0.03
% variation	- a / A	11.11 a / A	33.33 a / A	-22.22 a / A	- a / A	25.00 a / A	25.00 a / A	-25.00 a / A
I	82.19 ± 5.58	83.32 ± 4.88	71.81 ± 2.99	91.01 ± 10.03	81.50 ± 5.75	83.51 ± 4.25	73.91 ± 3.30	93.26 ± 11.49
% variation	- ab / AB	1.37 a/A	-12.63 b / A	10.73 ab / A	- a / A	2.47 a / A	-9.31 a / A	14.43 a / A
[MP] Polymer	1%				1%			
	L	PE	PA	PS	L	PE	PA	PS
A	143.68 ± 13.66	96.41 ± 11.64	68.12 ± 17.33	132.43 ± 7.33	106.5 ± 11.8	59.74 ± 9.34	41.15 ± 8.37	105.54 ± 10.37
% variation	- a / B	-32.90 b / A	-52.59 c / B	-7.83 a / A	- a / B	-43.91 b / A	-61.36 c / B	-0.90 a / A
k	0.07 ± 0.01	0.11 ± 0.01	0.28 ± 0.14	0.08 ± 0.01	0.08 ± 0.01	0.1 ± 0.02	0.32 ± 0.14	0.07 ± 0.01
% variation	- a / A	57.14 b / A	300.00 ab / A	14.29 ab / A	- a / A	25.00 a / A	300.00 a / A	-12.50 a / A
I	86.42 ± 2.24	76.15 ± 1.45	66.82 ± 0.97	75.41 ± 2.39	88.12 ± 3.31	76.90 ± 3.40	65.95 ± 1.79	80.37 ± 2.91
% variation	- a / A	-11.88 b / A	-22.68 c / A	-12.74 bc / B	- a / A	-12.73 b / B	-25.16 c / A	-8.79 ab / B
[MP] Polymer	10%				10%			
	L	PE	PA	PS	L	PE	PA	PS
A	142.80 ± 7.29	122.11 ± 6.90	76.52 ± 6.90	122.08 ± 10.69	80.76 ± 3.44	88.61 ± 5.14	54.16 ± 4.35	67.52 ± 3.32
% variation	- a / B	-14.49 b / B	-46.41 c / B	-14.51 b / B	- a / A	9.72 a / B	-32.94 c / AB	-16.39 b / B
k	0.09 ± 0.02	0.11 ± 0.02	0.09 ± 0.03	0.08 ± 0.01	0.12 ± 0.02	0.1 ± 0.02	0.10 ± 0.02	0.11 ± 0.02
% variation	- a / A	22.22 a / A	0.00 a / A	-11.11 a / A	- a / B	-16.67 a / A	-16.67 a / A	-8.33 a / A
I	74.82 ± 2.24	77.76 ± 2.20	73.74 ± 4.03	73.75 ± 2.64	71.35 ± 1.75	78.95 ± 2.25	74.32 ± 3.33	68.61 ± 2.13
% variation	- a / B	3.93 a / A	-1.44 a / A	-1.43 a / B	- a / B	10.65 b / AB	4.16 ab / A	-3.84 a / C

II.3.4. Fertility

II.3.4.1. Embryonic development time and hatching rate

The hatching rates and the embryonic development times are given in Table 7. No differences were detected in the hatching rate either regarding an effect of the polymer type or the effect of its concentration. For the embryonic development time, no concentration effect had been detected, and only a slight increase was detected at 10 % between PE (12.06 ± 0.32 d) and PS (13.06 ± 0.31 d, $p = 0.03$) exposed snails.

II.3.4.2. Fertility parameters

The modeled fertility curves for the control and the exposed animals are given in supplementary material (Figure A.2., Annex 8) and the values of the estimated parameters are provided in Table 6. The results show that the snail fertility was impacted by the exposure to MPs. At 0.1 % exposure concentration, neither the number of hatched eggs (A), nor the fertility constant (k), nor the date of the hatching peak (I) were affected by the modality. At 1 %, exposure to PE particles led to less hatchlings (-44%, $p < 0.01$) than L or PS exposed animals. Polyamide led to the heaviest impacts. Exposed animals not only produced less hatchlings than L (-61.36 %), PE or PS groups ($p < 0.01$) but also the egg hatching peak occurred earlier in PA (-22 days), followed by PE (-11 days) and PS (-8 days) vs L (88.12 ± 3.31 d, $p = 0.01$). At 10 % MP in food, the number of hatched eggs per snails was lower in PA-exposed animals compared to L (-32.94 %) and PE ($p < 0.01$) or PS ($p = 0.02$). In the same way, snails produced less hatchlings when exposed to PS particles (67.52 ± 3.32 hatch) compared to L (-16.39 %) and PE ($p < 0.01$). In addition, the exposure to PE also delayed the time to reach the hatching peak in comparison to L ($p = 0.01$) or PS ($p < 0.01$). The MP exposure concentration also appears to modulate the snail responses. Hence, the exposure to 10% PE increased the number of hatchlings ($p < 0.01$) but also shortened the time to reach the hatching peak ($p < 0.01$) in comparison to 0.1 % or 1 %. When exposed to PA, less hatches occurred at 1 % vs 0.1 % ($p = 0.01$). Concerning PS, on one hand the number of hatchlings decreased at 10 % exposure in comparison to 1 % ($p < 0.01$) and on the other hand, the hatching peak occurred earlier at 1 % group vs 0.1 % (-13 d, $p < 0.01$) and even earlier at 10 % (-25 d, $p < 0.01$).

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Table 7: Hatching rate (in %) and embryonic development time (in d) for control and MP exposed modalities at each concentration, with the associated percentage of variation for each MP modality compared to the control in each concentration. The letters represent the significant differences for each parameter between modalities for the same concentration.

MP concentration Polymer	0.10%			
	L	PE	PA	PS
Hatching rate	57.69 ± 7.65	69.94 ± 7.65	67.00 ± 7.65	69.18 ± 7.65
% variation	-	21.23	16.14	19.92
	a	a	a	a
Embryonic development time	11.69 ± 0.65	11.95 ± 0.66	12.52 ± 0.66	12.83 ± 0.63
% variation	-	2.22	7.10	9.75
	a	a	a	a
MP concentration Polymer	1%			
	L	PE	PA	PS
Hatching rate	74.61 ± 5.96	58.58 ± 5.96	63.85 ± 5.96	73.19 ± 5.96
% variation	-	-21.49	-14.42	-1.90
	a	a	a	a
Embryonic development time	12.83 ± 0.57	12.26 ± 0.51	12.84 ± 0.60	12.31 ± 0.51
% variation	-	-4.44	0.08	-4.05
	a	a	a	a
MP concentration Polymer	10%			
	L	PE	PA	PS
Hatching rate	62.66 ± 6.54	71.76 ± 6.54	76.14 ± 6.54	60.14 ± 6.54
% variation	-	14.52	21.51	-4.02
	a	a	a	a
Embryonic development time	12.75 ± 0.33	12.06 ± 0.32	12.50 ± 0.33	13.06 ± 0.31
% variation	-	-5.41	-1.96	2.43
	ab	a	ab	b

II.3.5. Mortality

We also monitored the mortality over the experiment. The results are presented in Figure 17 and in supplementary material (Table A.3., in Annex 9) and show that snail mortality depends on the duration of exposure but also on the polymer type and its concentration in food. When exposed to 0.1 %, mortality progressively increased to reach around 13% after 112 days of exposure, without any differences between the modalities.

At the intermediate exposure concentration (1 %), no differences were found until the 4th month of exposure, where the highest mortality was observed for the control (22.2 ± 5.38 %) and the lowest for the PS exposed animals (2.78 ± 5.38 %, p = 0.04). At 10 %, differences in mortality between exposure modalities appeared at the 2nd month and persisted until the end

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of the experiment, with a higher mortality in control (16.70 - 25 % from 2nd to 4th month) vs PE (2.78 - 5.56 %) or PA (2.78 - 8.33 %) exposed snails.

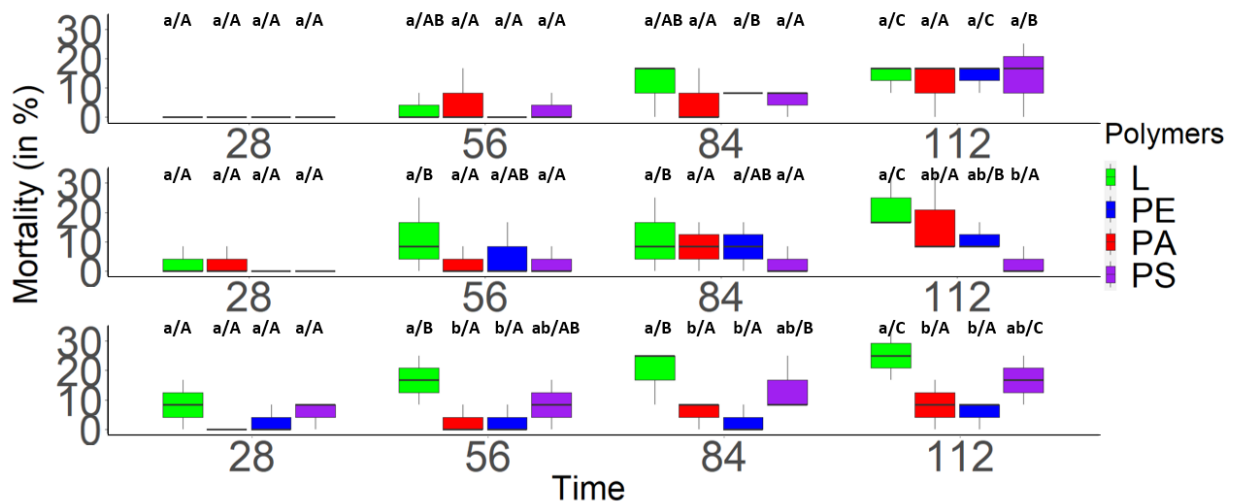


Figure 17: Snail mortality (in %) during the 4 months exposure to 0.1 % (upper panel), 1 % (middle panel) or 10 % (lower panel) of MP or lettuce (time in days). The lowercase letters represent the significativity for each parameter between modalities for the same concentration. The uppercase letters represent the significativity for each parameter between concentrations for the same modality.

II.3.6. Relations between growth, reproduction and mortality

We finally explored the relations between growth, reproduction and mortality during the experiment. The results are presented in Figure 18, and in the supplementary materials (Tables A.4 to A.6, in annex 10 to 12). As shown by curves in the Figure 18, the survival seemed to be more negatively impacted by reproduction for both the L and the MP modalities at 0.1 % (-0.008 to -0.019, $p < 0.001$, Table A.4., annex 10), the impact being lessened at the 1 % exposure (-0.0009 to -0.012, $p = 0.003$, Table A.4., annex 10) and at 10 % (-0.005 to -0.01, $p = 0.003$, Table A.4, annex 10) concentrations. The negative slopes suggest that when more eggs were laid by the animals, the more reproduction impacted survival. Whatever the exposure concentration, the effect of reproduction on animal survival seemed to be lower for PA vs the L ($p = 0.04$), the PE ($p = 0.0014$) or the PS ($p = 0.022$) modalities. Furthermore, the effect of the reproduction on mortality was no more significant for PA at 1 and 10 %. Concerning the effects of the concentration, the mortality was less impacted by reproduction in the 1 % ($p = 0.0132$) and the 10 % ($p < 0.001$) vs the 0.1 % modalities, whatever the polymer considered.

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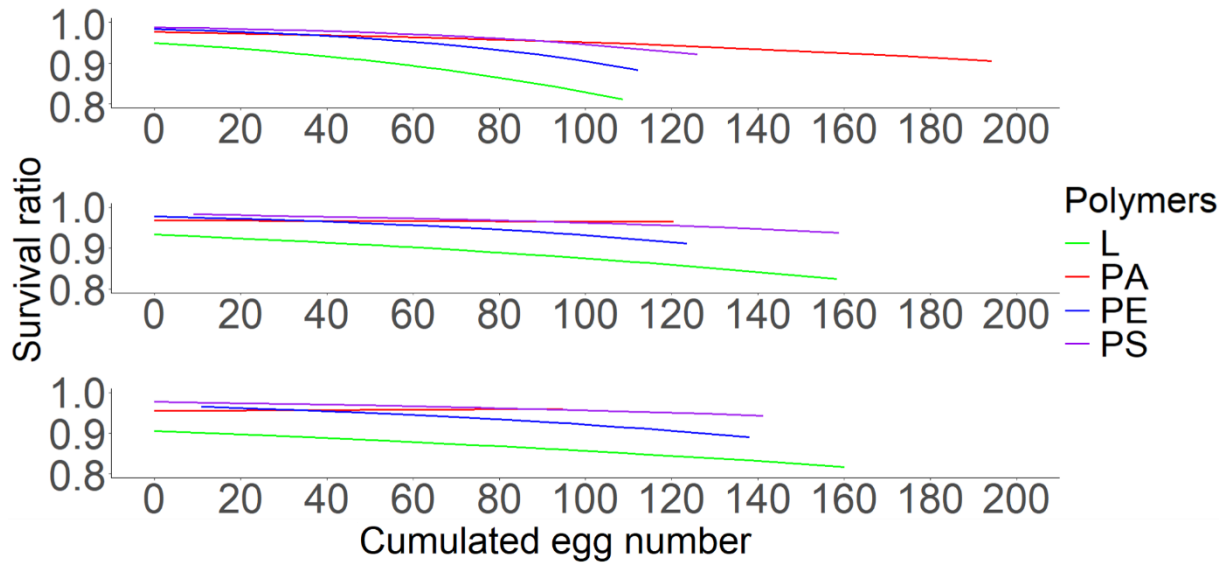


Figure 18: Snail predicted survival as a function of the number of eggs laid at 0.1 % (upper panel), 1 % (middle panel) or 10 % (lower panel) of MP or lettuce control groups

Concerning the effects of growth on survival (Table A.5., annex 11), we observed negative slope values for almost all the modalities, except for L at 1 % and for the PS exposure. The negative slopes could indicate that the bigger the animals, the more growth impacted survival. Whatever the concentration, the mortality appeared to be less impacted in PS than in PE ($p=0.03$) or PA ($p=0.02$), while no difference was found between PE and PA, or for L vs the other modalities. Concerning the concentration exposures, no differences were detected between the different exposure concentration on the effect of growth on mortality, whatever the polymer considered.

Finally, considering the relation between growth and reproduction, we did not observe significant relation between the two parameters, as the tested models were non-significant (Table A.6., annex 12).

II.4. Discussion

II.4.1. MP exposure can influence growth

Our study showed the influence of small-sized MP particles on the snail growth trajectories. At the lowest concentration (0.1%), a growth enhancement was observed for snails exposed to both PE, PA and PS (in comparison to the controls), while at higher concentrations, MPs

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(notably PE and PA) tended to lower the snail maximal mass. Several studies focused on the growth trajectories on a wide range of biological models, implying a whole range of responses, from a net weight gain in *Achatina reticulata* exposed to PET particles (De Felice et al., 2021), to a lack of MP effects for *Achatina fulica* snails fed from 0.014 to 0.71g.kg⁻¹ PET particles (Song et al., 2019) or no modification of growth in *Eisenia andrei* exposed to PE (62.5 to 1000 mg.kg⁻¹; Rodriguez-Seijo et al., 2017). Growth enhancement in snails has also already been seen by De Felice et al. (2021) in *A. reticulata* snails exposed to 1 and 10 % PET in food while such exposure concentrations led to a decreased body mass in our present work. However, De Felice et al. (2021) used another polymer, with bigger particle sizes mainly ranging between 100 and 1000 µm. Their results are consistent with the results of our previous work focusing on a lone polymer (PE), on larger particle sizes (d50 from 120 to 560 µm), highlighting the fact that the biological effects of MPs dependent on both their size and their concentration (Colpaert et al., 2022). At a low exposure concentration, “big” MPs (> 500 µm) may have facilitated the food items shredding inside the gastrointestinal tract of exposed animals, mechanically enhancing the assimilation and thus energy intake (Colpaert et al., 2022). As in the present study, this growth pattern was however reversed at higher MP concentrations in food, with exposed snails growing lesser than non-exposed ones. This kind of enhancement at low concentration followed by nefarious effect at higher concentration meets the definition of a hormetic effect (Jager et al., 2013). The causes may be multiple (Rix et al., 2022; Sun et al., 2021), from a false satiety caused by MP ingestion, or even a digestive tract blockage, as suggested for snails, springtails or nematodes (Y. Zhang et al., 2022), loss of carbonated biomass ingestion and thus energy depletion (Dissanayake et al., 2022), direct physical damages on the intestinal tract (Song et al., 2019) or via the triggering of oxidative stress and/or inflammation on intestinal tissues (Lei et al., 2018; Silva et al., 2021; Colpaert et al., 2022). Here, we assume a combination of physiological costs allocated to MP stress-induced management could explain the weight loss of exposed animals compared to control.

II.4.2. MP exposure negatively impact snail reproduction

Although the maximal number of animals sexually mature globally exceeded 90 % whatever the exposure modality, we evidenced the influence of small-sized MP particles on the snail

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reproduction dynamic. Indeed, in MP-exposed organisms, the sexual maturity was achieved sooner (up to almost 10 days), notably at low and medium exposure concentrations. From our knowledge, our study is one of the very few addressing the impact of MPs on the kinetic of acquisition of the sexual maturity on an invertebrate model. Among these rare studies, Sobhani et al. (2021) did not observed an effect of 0.01 to 0.5 % (w/w) PS in soils on the maturation time of *E. fetida* earthworms, while Cheng et al. (2023) observed a delayed time of pregnancy on *D. magna* challenged with PS particles. More data on vertebrates are available. For example, in aquatic ecosystems, Fernández-Míguez et al. (2023) had observed no effects of 1 % PE (0.3 to 0.6 mm) on the gonadal development during the sexual maturation of Atlantic cods. In terrestrial environments, a delay in the age of first oestrus in rats (Solleiro-Villavicencio et al., 2020) or an early puberty in humans (Sarkingobir et al., 2020), especially in women (Arrigo et al., 2023), were recently documented. These impacts in vertebrates are mainly linked in the literature to a chemical effect related to the additives contained in or coated on MPs, being estrogen-like chemicals (Yang et al., 2011) and exerting their endocrine disrupting effects through an action on the hypothalamic-pituitary axis (Ullah et al., 2023). As snails may be sensitive to endocrine substances (Druart et al., 2017), the precocity of the sexual maturity we observed could be associated with the release of endocrine disrupting compounds from MP ingested particles.

The fecundity was also impacted by the MP exposure, with some influence on the egg laying peak (*e.g.*, earlier at low PA concentration), but the most noticeable being a concentration-dependent decrease (for PA at 1 and 10 % vs 0.1 %) or increase (for PE at 10 % vs 0.1 or 1%) of the egg production for the MP-exposed snails, while embryonic development time and hatching rate were not disrupted. Thus, fertility, which is the result of both fecundity (the capacity for the adult snail to produce eggs) and hatchability (the capacity for the embryo to develop, showing the quality of the produced eggs), followed the same concentration-dependent patterns for PE and PA, all MP-exposed groups tending to have lowered fecundity and fertility than control as the concentration of exposure increased, with the effects depending on the type of MP. Several studies have explored the relationship between MP exposure and egg or offspring production, with no reproductive effect of PE (62.5 to 1000mg.kg⁻¹ soil) on *E. andrei* (Rodriguez-Seijo et al., 2017) or no effect of 7 to 60 % PE in litter on *L. terrestris* ability to produce cocoon (Huerta Lwanga et al., 2016), while Jimenez-Guri et

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al. (2021) observed more eggs produced for 1 % PE and marginally more eggs produced for 4 % PVC in exposed *D. melanogaster*. Other studies have demonstrated deleterious effects of MP, for example, Kumari et al. (2023) observed a dose-dependent decreased egg capsule production, juvenile production and hatching success in the aquatic snail *Physa acuta* exposed to PS-MP from 100 to 200 mg.L⁻¹. Similarly Cheng et al. (2023) found a lower number of progeny in *D. magna* exposed to 1mg.L⁻¹ PS MP, while Ju et al. (2019) observed on the soil springtail *F. candida* a concentration-dependent decrease of the reproduction rate with the increase of PE exposure. Kwak and An (2021), though not directly observing the reproduction rate of *E. andrei* earthworms, did bring a possible explanation of the deleterious effects of MP by observing a decrease in sperm density, an increased disorder of the germ cell arrangement and damages to sperm plasma membrane in seminal vesicles of the animals exposed to PE MP at 1000 mg.kg⁻¹. The same mechanism was also argued in several studies on vertebrates. Qiang and Cheng (2021) observed increased reactive oxygen species (ROS) in both male and female gonads of zebrafish exposed to 100 and 1000 µg.L⁻¹ PS MP (1µm), associated to an upregulation of mRNA-expression of apoptosis-related genes and a reduction of the basement membrane thickness in testis of the exposed animals. Testis histopathologic damages, associated to sperm malformations, necrotic spermatogenic cells and upregulation of apoptosis- and estrogen-receptors-regulating genes were also observed in mice fed 0.01 to 1mg.d⁻¹ 10 µm PS MP (Wen et al., 2023). A mechanism may be that once ingested, MPs, or their additives, may cross biological barriers and exert both physical and additive-related chemical damages on reproductive tissues. These additive-related effects are mainly mediated by oxidative stress and associated with an upregulation of prooxidant mediators, an inhibition of antioxidant defenses, and an upregulation of cell death pathways, proinflammatory pathways and cytokines (Urli et al., 2023). Such effects may eventually occur even if the MP internalization efficiency may be relatively low (under 0.05 % for MPs and under 10 % for nanoplastics; Ziani et al., 2023). The physical and chemical effect hypothesis may be reinforced by Zhao et al. (2023) who detected several MP particles (such as PE, PVC, PA, PS, PP and PET) of different sizes (mainly in the <50 µm range, but up to 200 µm) in human semen and in human gonads, and by Hu et al. (2024) who also recently found several high MP concentrations in dog (122.63 µg.g⁻¹) and human (328.44 µg.g⁻¹) testis. It is worth to be noted than in the latter study, all testis samples (dog as well as human ones) were MP contaminated.

II.4.3. MP exposure does not hinder snail survival, but does not protect snail

Overall, during the experiment, mortality rose more slowly for the MP exposed animals, and reached lower levels with increasing exposure to MPs, whatever the polymer considered. Several studies have addressed the MP impacts on invertebrate mortality. For example, Rodriguez-Seijo et al. (2017) observed no effect of PE on *E. andrei* mortality after a 28 days of exposure; the same tendency being observed by Sobhani et al. (2021) either on *E. fetida* subjected to PS MP (65-125 µm, 0.01 to 0.5 % w/w in soil) or on their offspring. Also, in agreement with their studies, we did not show increased mortality on the *C. aspersus* snails subjected to PE particles during 28 days (Colpaert et al., 2022). On the other hand, El Kholy and Al Nagggar (2023) observed an increased mortality in *D. melanogaster* exposed 7 days to PS MP, as well as a decreased starving resistance in their male offspring, while Ju et al. (2019) observed a decreased survival in springtails *F. candida* exposed to 1 % PS, and Cheng et al. (2023) showed a lowered survival rate in *D. magna* exposed 21 days to PS (5 µm to 0.5 µm). In addition to the type, the size and the concentration of the MP used, the duration of exposure and the consideration of successive biological endpoints may also be of importance. In this experiment, no detrimental effect of MP exposure on survival have been detected. On the contrary, MP-treated snails died less and later than the controls. However, survival is an apical endpoint which depends on the energy budget allocated notably to maintenance, which is one of the main physiological processes in animals along with growth, maturity and reproduction (Kooijman, 2009). In such circumstances, and according to the high number of eggs produced per mature animal, the hypothesis retained in this study to explain, at least partly, the differences in mortality is a trade-off in the energetic allowance of the animal. Here, in the control animals, the energy budget allowance could benefit to a high reproduction rate. This high reproduction rate could then potentially lead to a depletion of the energetic reserves of the animals, causing death by exhaustion. In the case of MP exposed animals, the MP ingestion may have led to possible direct physical damages on tissues, and/or on the snail microbiome affecting the food assimilation efficiency, thus leading to less energy available for the animals. Furthermore, prooxidative and inflammation processes (or a combination of both) may also potentially be impacted by MPs, causing energetic cost for the animals (Silva et al., 2021; Wright et al., 2013). Our hypothesis is that the MP effects may have triggered a shift in the energetic trade-off of the animals, from reproduction to maintenance of the body

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function to compensate the effects of the MPs, especially at higher concentrations, which could explain the lowered reproduction rates. Such reproductive perturbations may also be enhanced by released additive chemicals (endocrine disruptive compounds) from the MPs. Finally, these trade-offs potentially led to animals having a lesser egg production (without significant effects on the quality of the produced eggs) and a decreased body mass, in order to increase their fitness. Such trade-offs finally eventually prevented them to deplete their energetic reserves by investing in reproduction, thus explaining the higher survival rate compared to the control animals. Such hypothesis has been investigated by looking at the relations between the survival, the reproduction and the mass. Overall, the survival of the MP exposed animals seemed to be negatively affected by reproduction in all the modalities. More in details, the impacts of reproduction on survival in exposed animals was lower than in non-exposed ones, with a lessened impact as the exposure concentrations increased. This relation between the animal survival and reproduction seems to indicate a preservative effect of the reproduction on survival, which could accredit the assumption of energetic trade-offs in exposed animals. Only a few studies have already addressed such effects of microplastics in animals. In agreement with our results, Wang et al. (2021) as well as Tourinho et al. (2022) have also observed such trade-offs between basal maintenance, growth and reproduction under microplastic-induced stress situation in *Oryzias melastigma* and under combined AgNP, AgNO₃ and MP fibers in *Daphnia magna*. Our study highlighted interesting bundle of evidences concerning trade-offs triggered by MP exposure, however, further studies are necessary to deepen our understanding of these phenomena. Even if in this study, the described effect could be linked to energetic trade-offs, such effects could also be linked (at least partially) to endocrine disruption caused by additives leached from the MPs. Thus, future studies should address energy reserve (protein, carbohydrates and lipid content) and energy consumption analyses (electron transport activity) to assess the presence or absence of energetic trade-offs, in order to decipher the about which mechanisms between energetic trade-offs, endocrine disruptions, or a combination of both could explain this phenomenon. Furthermore, such future studies should also incorporate oxidative stress and transcriptomic analysis to further develop the underlying mechanisms.

II.5. Conclusion

After a 4-month exposure to PE, PA or PS particles in food, *C. aspersus* snails showed polymer dependent responses on a wide variety of assessed endpoints. If the lowest concentration tend to increase the growth compared to the control suggesting an hormetic effect of the 3 MP, overall a concentration-dependant decrease of the snail growth is observed.

Furthermore, while not affecting the percentage of sexually mature individuals, MP exposure modified the temporality of the acquisition of the sexual maturity and lowered the number of produced eggs and then the number of produced progenies. The general trend was toward the acquisition of an earlier maturity acquisition in the low and middle contamination groups, with no significant impact on the hatching rate or the embryonic time development, thus without following the concentration-dependent trend observed for growth, depending on the type of MP. This decrease of egg production was not accompanied by a decrease in the quality of the eggs produced. A probable cause could be pro-oxidative and/or pro-inflammatory processes triggered by the MP exposition. Finally, these pro-inflammatory processes affected the energetic tradeoffs of the individuals, leading to an increased energy invested in the maintenance of the organism, lowering the mortality otherwise imputed to the reproduction process. These mechanistic processes still remain to be elucidated, since the fertility did not follow a concentration dependent effect in our experiment. This study shows at first that long-term, life-cycle assessment are relevant to better understand both the effects of the MPs on the tested animals, and the possible underlying mechanistic explanations, by recouping a wide variety of endpoints. Our work also shows that even not directly affecting the survival of exposed individuals, these contaminations by MPs may lead to more subtle effects, impacting the animals at an individual scale, with possible broader deleterious outcomes for the populations, as well as their ecosystems, in natural habitats.

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Chapitre III. Etude de la capacité des microplastiques à moduler la disponibilité environnementale et la biodisponibilité d'éléments traces métalliques.

Titre en anglais: The effects of polystyrene microparticles on the environmental availability and bioavailability of As, Cd and Hg in soil for the land snail *Cantareus aspersus*

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Résumé

Les contaminations combinées des environnements terrestres tels que les agroécosystèmes par les métalloïdes (MEs) et les microplastiques (MPs) est un problème environnemental majeur. Une fois que les MPs pénètrent dans le sol, ils peuvent interagir avec les MEs et modifier leur disponibilité environnementale, leur biodisponibilité environnementale, et leurs potentiels effets toxiques envers le biote. Bien que des efforts de recherche ont été fait pour décrire les mécanismes sous-jacents dirigeant les interactions entre MEs et MPs, les effets des MPs sur la biodisponibilité des MEs envers les mollusques terrestres n'a pas encore été documentée. Pour combler cette lacune, nous avons exposé des escargots terrestres (*Cantareus aspersus*) à différentes combinaisons de concentrations en polystyrène (PS) avec de l'arsenic (As), du cadmium (Cd) et du mercure (Hg). En utilisant des approches cinétiques, nous avons ensuite évalué la disponibilité environnementale de l'As, du Cd et du Hg dans les sols après trois semaines d'équilibration et leur biodisponibilité environnementale dans l'escargot après 4 semaines d'exposition. Les résultats ont montré que bien que la disponibilité environnementale soit influencée par la concentration en ME, les effets du PS étaient limités. Bien qu'une augmentation de la disponibilité environnementale de l'As ait été observée à la plus haute concentration d'exposition au début de l'expérimentation, les processus de vieillissement du sol ont conduit à une adsorption rapide des MEs dans le sol peu importe la concentration en PS. Concernant le transfert aux tissus de l'escargot, la bioaccumulation en ME était concentration-dépendante, mais n'était pas modifiée par la concentration en PS dans le sol. Néanmoins, les approches cinétiques ont montré une augmentation de la biodisponibilité environnementale (taux d'absorption) de l'As (de 2 à 2,6 fois) et du Cd (1,6 fois), ainsi que de leur excrétion (2,3 à 3,6 fois pour l'As, 1,8 fois pour le Cd) à faible concentration en PS, sans qu'il n'y ait d'effet pour le Hg. Cependant, ces effets n'étaient plus observables à la plus haute concentration en PS, à cause de l'augmentation de la bioaccessibilité des MEs dans le tractus digestif de l'escargot. La généralisation de tels effets hormétiques et l'identification des mécanismes précis impliqués nécessitent de futurs efforts de recherche pour approfondir nos connaissances sur le comportement des MEs médiés par les MPs dans de tels scénarii de co-occurrence.

Abstract

The combined contamination of terrestrial environments such as agroecosystems by metal(loid)s (MEs) and microplastics (MPs) is a major environmental issue. Once MPs enter soils, they can interact with MEs and modify their environmental availability, environmental bioavailability, and potential toxic effects on soil biota. Although research efforts have been made to describe the underlying mechanisms driving MP and ME interactions, the effects of MPs on ME bioavailability in terrestrial Mollusca have not yet been documented. To fill this gap, we exposed the terrestrial snail *Cantareus aspersus* to different combinations of polystyrene (PS) and arsenic (As), cadmium (Cd), or mercury (Hg) concentrations. Using kinetic approaches, we then assessed the variations in the environmental availability of As, Cd or Hg in the soil after three weeks of equilibration and the environmental bioavailability of As, Cd or Hg to snails after four weeks of exposure. The results showed that while environmental availability was influenced by the total ME concentration, the effects of PS were limited. Although an increase in As availability was observed for the highest exposure concentrations at the beginning of the experiment, the soil ageing processes led to rapid adsorption in the soil regardless of the PS particle concentration. Concerning transfers to snail tissues, ME bioaccumulation was ME concentration-dependent but not modified by the PS concentration in the soils. Nevertheless, the kinetic approaches showed an increased As (2- to 2.6-fold) and Cd (1.6-fold), but not Hg, environmental bioavailability (uptake rates) or excretion (2.3- to 3.6-fold for As, 1.8-fold for Cd) at low PS concentrations. However, these impacts were no longer observable at the highest PS exposure concentrations because of the increase in the bioaccessibility of MEs in the digestive tract of the snails. The generalization of such hormetic responses and the identification of the precise mechanisms involved here necessitate further research to deepen our understanding of the MP-mediated behaviour of MEs in co-occurring scenarios.

III.1. Introduction

Since the mid-1950s, plastics have become widespread in the products we use in our modern societies, with annual production ranging from almost 2 million metric tons in 1950 to 391 million metric tons in 2021 (Plastics Europe, 2022; Zhang et al., 2020). Plastic production, use and disposal management, and mismanagement lead to the risk of plastic leakage into the environment (Helm et al., 2023). Even if the collective conscience is now aware of the hazards posed by plastic use on our environment and global policy actions have been proposed to reduce the effects on plastic pollution, these policies are globally insufficient and lack specific measurable targets to reduce plastic contamination effectively worldwide (Diana et al., 2022). Plastic waste can be recycled (9% of plastic waste), incinerated (12% of plastic waste), or dumped, landfilled or littered into the environment (UNEP, 2021), leading to global soil and water contamination. As plastics are produced, primarily used and discarded in terrestrial environments, continental environments are presumably 4- to 23-fold more contaminated than oceans are (Horton et al., 2017). Once in the environment, plastic waste can undergo weathering processes through abiotic or biotic actions. These weathering processes are susceptible to changes in surface physicochemical properties (Barnes et al., 2009) and can lead to fragmentation rather than degradation (de Souza Machado et al., 2018; Song et al., 2017) or the formation of microplastics (MPs). MPs are plastic particles measuring between 5 mm and 0.1 μm in size (Frias and Nash, 2019). In the terrestrial environment, agricultural lands are particularly at risk of MP pollution, because they can be contaminated by plastic items and MP particles through aerial deposition (Dris et al., 2017), plastic and MP pollution due to plastic waste mismanagement, the use of plastic agricultural mulch films (Ren et al., 2021; Scarascia-Mugnozza et al., 2012; Xu et al., 2022) and the use of MP-contaminated organic waste products (sludge, compost, digestates) as fertilizers (Bellasi et al., 2020). Microplastics can also act as carriers for many co-occurring organic or inorganic pollutants, including pesticides, hydrocarbons, pharmaceuticals and metal(loid)s (MEs; Kinigopoulou et al., 2022). These co-existing contaminants are likely to occur in natural environments and agroecosystems, where their co-occurrence is susceptible to changes in contaminant behaviour, environmental availability (*i.e.*, the fraction of contaminants physicochemically driven by desorption processes that are potentially available to organisms) or bioavailability (*i.e.*, the fraction of environmentally available compounds that an organism takes up through

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physiologically driven processes) and hence changes its potential toxicity (ISO 17402, 2008; Xiang et al., 2022). Co-exposure can lead to various effects on both environmental availability and bioavailability towards biota, with potential desorption of MEs from MPs to animals before and/or after ingestion, increasing ME accumulation in animals (An et al., 2023; Bradney et al., 2019). Several studies have been conducted to gain further insight into the possible effects of this co-exposure on ME accumulation in soil invertebrates, *e.g.*, in earthworms, enchytraeids, springtails or isopods (M. Li et al., 2023; Selonon et al., 2021). The study of MP effects on soil biota is still challenging, with MPs having varying, and even contradictory, effects according to the polymer type and the species under consideration (Ji et al., 2021; M. Liu et al., 2023; Rodrigues et al., 2023). However, no studies have investigated the effect of MP and ME co-contaminations on terrestrial gastropods.

In this study, we aimed to assess the impacts of co-exposure to polystyrene (PS) particles on the behaviours and fates of different MEs in soils. The main questions this study aimed to answer were as follows: 1) Can PS particles impact the environmental availability of As, Cd and Hg in soils? 2) Do these MPs modulate the environmental bioavailability and bioaccumulation of MEs in snails? 3) What is the influence of the PS exposure concentration? We then used a combination of chemical methods and biological approaches using the terrestrial snail *Cantareus aspersus*, a relevant, recognized and standardized bioindicator of soil quality (de Vaufleury, 2015; ISO 15952, 2018). The studied MEs were chosen because they are naturally occurring geogenic contaminants that may become pollutants in the terrestrial environment due to various anthropogenic activities (Fernandes et al., 2021; Garelick et al., 2009; Mora et al., 2023; Six and Smolders, 2014) as well as their different behaviours in the soil, transfer potential and toxicity towards biota. Polystyrene plastic was used in this study because it is extensively used in human activities (industry, agriculture), has been found in large quantities in composts used as fertilizers (up to 35 % of the MPs retrieved in sewage sludges, Baho et al., 2021; Ng et al., 2018), and can therefore heavily contaminate agroecosystems. Polystyrene particle ingestion can also exert deleterious effects on terrestrial biota, including oxidative stress, histopathological changes, DNA damage, neurodevelopmental toxicity, and immune and metabolic deregulation (Qiao et al., 2022). The PS size used here (80 μm) was chosen because it is representative of a large proportion of MP sizes in agroecosystems (Feng et al., 2021; Zhou et al., 2019).

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The specific objectives of this study were to assess the impact of PS particles on the environmental availability of As, Cd and Hg in the soil and their environmental bioavailability and bioaccumulation in *C. aspersus* snails. To this end, the environmental availability of MEs was assessed via the equilibration kinetics of their CaCl₂-extractible concentration at 0, 3 and 7 weeks after soil contamination. To determine the dynamic physiological processes that drive ME fate in snails over a 4-week exposure, the bioavailability of MEs to *C. aspersus* was evaluated by their uptake rates. The snail ME excretion rates were also determined. The steady-state concentration, *i.e.*, bioaccumulation, resulting from uptake and excretion was also characterized.

III.2. Material and methods

III.2.1. Soil contamination

The soil used in this experiment was a soil from a scientific-usage parcel on organic residual material (Système d'Observation et d'Expérimentation pour la Recherche en Environnement sur les PRO; SOERE-PRO) from agricultural origin in the Paris region, coming from a non-amended parcel in the QualiAgro experimental site. The QualiAgro experiment is located at Feucherolles in northwestern France (35 km west of Paris; 48°52' N, 1°57' E, alt 150 m), with an oceanic climate, a mean rainfall of 594 mm year⁻¹ and a mean annual temperature of 10.8 °C. The soil is a Luvisol containing 16% clay, 78% silt, and 6% sand on average. The pH of the parcel is 6.5 and the organic matter (OM) content is 17.6 g.kg⁻¹ soil (Pérès et al., 2011; Sadet-Bourgeteau et al., 2018). The microplastic particles used in this experiment were spherical-shaped polystyrene microparticles (PS) with a mean diameter of 80.09 ± 1.07 µm intended for industrial use. The metals used were arsenobetaine (C₅H₁₁AsO₂; Arsenobetaine purum p.a., ≥95.0%), dimethyl arsenic acid (= cacodylic acid: C₂H₇AsO₂; ≥99.0%), sodium meta-arsenite (NaAsO₂; ≥90%), sodium arsenate dibasic heptahydrate (Na₂HAsO₄, 7H₂O; ≥98.0%), cadmium chloride (CdCl₂; 99.99% trace metals basis), mercury (II) chloride (HgCl₂; ACS reagent, ≥99.5%) and methylmercury (II) chloride (CH₃HgCl; PESTANAL[®], analytical standard). The extraction reagent used in the experiment was calcium chloride (CaCl₂, 2H₂O; ReagentPlus[®], ≥99.0%). All the metals and the calcium chloride were purchased at Sigma-Aldrich (Sigma-Aldrich Chimie SARL, 80 Rue de Luzais, L'Isle-d'Abeau Chesnes – BP701, 38297 Saint-Quentin-Fallavier Cedex, France) and dissolved in demineralised water.

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After soil sieving (2 mm mesh sieves) and drying at 37°C in an oven, the soil was contaminated with different As, Cd, Hg and PS concentrations in liquid phase in ultrapure water, as shown in table I. The soil used in this experiment was topsoil from a scientific-usage parcel on organic residual material (Système d'Observation et d'Expérimentation pour la Recherche en Environnement sur les PRO; SOERE-PRO), which was obtained from a non-amended parcel at the QualiAgro experimental site. The QualiAgro experiment is located at Feucherolles in northwestern France (35 km west of Paris; 48°52' N, 1°57' E, alt 150 m), which has an oceanic climate, a mean rainfall of 594 mm year⁻¹ and a mean annual temperature of 10.8 °C. The soil type is a hortic glossic Luvisol containing 16% clay, 78% silt, and 6% sand on average. The pH of the parcel is 6.5-7, and the organic matter (OM) content is 17.6 g.kg⁻¹ soil (Pérès et al., 2011; Sadet-Bourgeteau et al., 2018).

The MP particles used in this experiment were spherical-shaped PS with a mean diameter of 80.09 ± 1.07 µm, and they were intended for industrial use (3D printing). More precisely, the polymer used in this study was Primecast® 101 PS (by EOS GmbH), which is a commercial PS powder used as feedstock in the production of lost patterns for casting processes (the SEM images of the particles used here are shown in Figure S.1, in Annex 13). Some properties of this material are given in (Özer et al., 2020). For As and Hg, the total concentrations were shared between different organic and inorganic species, as defined in Table S.1 (Annex 14), and they corresponded to the percentage of ME species retrieved from different soils to reflect the forms of MEs in the agricultural environment (Davis et al., 1997; Duester et al., 2005; Geislinger et al., 2002; Hutton et al., 2005; Remy et al., 2006). The ME chemical forms used here were arsenobetaine (C₅H₁₁AsO₂; Arsenobetaine purum p.a., ≥95.0%), dimethyl arsenic acid (= cacodylic acid: C₂H₇AsO₂; ≥99.0%), sodium meta-arsenite (NaAsO₂; ≥90%), sodium arsenate dibasic heptahydrate (Na₂HAsO₄, 7H₂O; ≥98.0%), cadmium chloride (CdCl₂; 99.99% trace metals basis), mercury(II) chloride (HgCl₂; ACS reagent, ≥99.5%) and methylmercury(II) chloride (CH₃HgCl; PESTANAL®, analytical standard). All the MEs were purchased from Sigma–Aldrich (Sigma–Aldrich Chimie SARL, Saint-Quentin-Fallavier, France).

Following soil sieving at 2 mm and oven-drying at 37 °C, the soil was prepared to reach three ME and three PS exposure concentrations (Table 8). For the MEs, the first exposure concentrations corresponded to the geochemical background (no spike), the second corresponded to regulatory guidance values of surface soil contamination (Jennings, 2013),

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and the last concentrations corresponded to more extreme exposure scenarios (approximately 2.5 times the regulatory values). For PS, we investigated three exposure concentrations, 0 (no plastic addition), 0.01% (w/w), which corresponds to the order of magnitude (mg/kg) of PS in amended European soils (Büks and Kaupenjohann, 2020), and 1% (w/w) was used as the worst-case exposure scenario.

Table 8: Polystyrene (PS) and total concentrations of As, Cd and Hg used in the experiment.

PS As Measured concentration ($\mu\text{g}\cdot\text{g}^{-1}$)	0			0.01			1		
	0	50	100	0	50	100	0	50	100
	7.54 ± 1.20	43.41 ± 3.17	94.84 ± 3.39	7.01 ± 0.54	41.84 ± 2.95	98.08 ± 17.58	7.14 ± 0.30	41.13 ± 2.78	90 ± 6.56
PS Cd Measured concentration ($\mu\text{g}\cdot\text{g}^{-1}$)	0			0.01			1		
	0	10	20	0	10	20	0	10	20
	0.27 ± 0.03	8.80 ± 0.95	19.50 ± 0.52	0.25 ± 0.02	8.99 ± 1.36	17.47 ± 1.66	0.24 ± 0.01	9.38 ± 1.05	17.75 ± 0.72
PS Hg Measured concentration ($\mu\text{g}\cdot\text{g}^{-1}$)	0			0.01			1		
	0	2	5	0	2	5	0	2	5
	0.08 ± 0.004	1.24 ± 0.09	3.89 ± 0.36	0.08 ± 0.005	1.12 ± 0.18	3.43 ± 0.05	0.08 ± 0.002	1.21 ± 0.24	3.55 ± 0.24

To reach these exposure concentrations, the appropriate amounts of ME salts and/or PS particles were dissolved or suspended in a suitable volume of demineralized water and progressively added to the dry soil (1 kg) to reach 30% of the water holding capacity. The soils were then thoroughly homogenized and stored in glass containers under experimental conditions (20 ± 1 °C; 80% relative humidity, 18/6 h light/dark cycle) for seven weeks. The total ME concentrations in the soils were measured immediately after spiking. Soil samples (0.5 g, freeze-dried) were mineralized in 2 mL of HNO_3 and 5 mL of HCl (Optima grade, Sigma–Aldrich Chimie SARL) in a Digiprep™ apparatus (SCP Science; Quebec, Canada). The solutions were filtered (0.45 μm), and the volumes were adjusted to 50 mL with ultrapure water and subjected to ICP–AES (iCAP 6500, Thermo Fisher, Illkirch-Graffenstaden, France). The

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reliability of the analysis was determined using a certified reference material (Loamy Clay-2 certified soil, Sigma–Aldrich), with a mean recovery rate of $94 \pm 5\%$ for the tested MEs.

Humidity was then fixed at 30 % of the water holding capacity before homogenization and 21 days ageing in glass containers at room temperature. The first exposure concentration corresponds to the non-contaminated soil (no plastic addition, metal concentrations corresponding to the geochemical background measured in ICP-MS before the experiment, certified reference material: Loamy Clay-2 certified soil, mean recovery rate: $93.8 \pm 4.7\%$ for the tested metals). The second exposure concentration corresponds for PS to concentrations retrieved in European amended soils (Vollertsen, 2017), and for the metals to reference concentrations defined by the Finnish environment ministry, and used by the UNEP. The third exposure concentration correspond to the later exposure, multiplied by 2 to 2.5 for metals and by 100 for PS. For As and Hg, total concentrations were shared between different organic and inorganic species, as define in the table II, and corresponding to the percentage of metal species retrieved in different soils, to better reflect the behavior of metals in the agricultural environment (Davis et al., 1997; Duester et al., 2005; Geiszinger et al., 2002; Hutton et al., 2005; Remy et al., 2006).

III.2.2. Soil ageing and environmental availability

Soil samplings were performed just after spiking (referred to as t_0) and 3 and 7 weeks after spiking (referred to as $t+3$ and $t+7$, respectively). At each sampling time, the different ME environmental availabilities were assessed using 0.01 M CaCl_2 (CaCl_2 , $2\text{H}_2\text{O}$; ReagentPlus®, $\geq 99.0\%$; Sigma–Aldrich) extractions. This is a neutral salt (cation exchanger) recommended by the International Organization for Standardization to estimate the soluble and easily exchangeable fraction in soils (ISO 22190, 2020), and it corresponds to the fraction that is potentially available to the biota. In brief, after 2 hours of shaking (10 rpm) at a 1:10 (m/v) ratio of freeze-dried soil (3.5 g) and 0.01 M CaCl_2 solution (35 mL), the extract was separated from the solid residue by centrifugation at $3000 \times g$ for 10 min. Then, 10 mL of the supernatant was filtered through a cellulose acetate disk filter ($0.45 \mu\text{m}$) to avoid PS particle contamination of the collected supernatant. The extract was finally acidified using HCl (0.5%) to prevent adsorption on the storage vessel and growth of bacteria, and it was stored at 4°C until ME analysis. The ME concentrations in the extracted solutions were determined by ICP-MS (Icap

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RQ ICP-MS, Thermo Fisher Scientific). The reliability of the analysis was determined using a certified reference material (certified water, LGC6027, LGC standards, Molsheim, France) with a mean recovery rate of $106 \pm 7\%$ for the tested MEs.

III.2.3. Snail exposure and environmental bioavailability

The snails used here ($n=360$) were sub-adult *Cantareus aspersus* (anc. *Helix aspersa*, syn. *Cornu aspersum*) snails (aged two months and weighing 4.54 ± 0.47 g) that were obtained from our laboratory culture facility. The animals were bred in glass containers under controlled conditions according to Gomot-De Vaufleury (2000) with a temperature of 20 ± 1 °C, 80% relative humidity, and an 18/6 hour light/dark period and were fed *ad libitum* with snail flour (vegetal flour, Marion®; SAS aliment Marion, Saint Jean sur Veyle).

After 3 weeks of equilibration, the snails were exposed to the different treatments in glass containers (25x12x7 cm per box, 2 boxes per device) filled with 2 cm of soil (300 g wet weight) for a 28-day exposure period (10 snails per replicate, 3 replicates per modality). The individuals were maintained under the culture conditions described above and fed fresh organic lettuce three times a week in glass Petri dishes ($0.5 \text{ g.snail}^{-1}.\text{d}^{-1}$). After 3 weeks of equilibration, the snails were exposed to the different treatments in glass containers (25x12x7 cm per box, 2 boxes per device) filled with 2 cm of soil (300 g wet weight) for a 28-day exposure period (10 snails per replicate, 3 replicates per modality). The individuals were maintained under the culture conditions described above and fed fresh organic lettuce three times a week in glass Petri dishes ($0.5 \text{ g.snail}^{-1}.\text{d}^{-1}$). Lettuce samples were freeze-dried and mineralized (0.20 ± 0.01 g) using 3.5 mL HNO_3 and 1 mL H_2O_2 in a Digiprep™ apparatus (SCP Science, Quebec, Canada). The volume was adjusted to 25 mL in ultrapure water before filtration (1 μm), and ME dosing was performed via ICP-MS (Icap RQ ICP-MS, Thermo Fischer Scientific). The reliability of the analysis was determined using a certified reference material (INCT-OBTL5 Oriental Basma Tobacco Leaves, LGC standard, Molsheim, France), with a mean recovery rate of $104 \pm 9\%$ for the tested MEs.

All the individuals were weighed on days 0, 2, 5, 7, 14, 21 and 28 of the exposure periods (from the 3rd to the 7th week after soil contamination) for growth assessment. At each of these time points, an individual in each replicate was randomly sampled and then sacrificed by freezing at -80 °C for further ME analysis. Following defrosting, the animals were dissected in a specific

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P2-type laboratory with non-plastic material (*i.e.*, glass dissection boards, stainless steel pliers and scissors, glass tubes for organ collection, cotton laboratory coats and shoe covers), and the digestive glands were retrieved for ME analyses. The digested gland samples were freeze-dried and mineralized (0.25 ± 0.010 g) using 1.75 mL HNO₃ (Optima grade, Sigma–Aldrich Chimie SARL) in a Digiprep™ apparatus (SCP Science; Quebec, Canada). The volume was adjusted to 12.5 mL in ultrapure water before filtration (0.45 μm), and ME dosing was performed via ICP-MS (Icap RQ ICP-MS, Thermo Fisher Scientific). The reliability of the analysis was determined using a certified reference material (TORT-3 lobster hepatopancreas, LGC standard, Molsheim, France), with a mean recovery rate of $118 \pm 13\%$ for the tested MEs.

III.2.4. Statistical analyses

To reach statistical prerequisites (normality, homoscedasticity), all the data were log(x+1)-transformed.

The influence of total ME and PS concentrations on ME-extractible concentrations and extraction yields in soils were checked using linear mixed effect models (lme, lme4 R package), integrating the exposure modalities as fixed effects and the replicates as random effects.

The ME concentrations in the soils over time were modelled using a first-order kinetic model, following equation 1:

$$C(t) = C0 \times e^{(-k0 \times t)}$$

where C(t) is the extractible concentration (in μg·g⁻¹) at time t, C0 is the initial ME concentration (in μg·g⁻¹), k₀ is the rate constant of decrease in the extractability (in d⁻¹), and t is the time (in d).

The modelled k₀ was also used to calculate the ME extractability half-lives in the soil (in d), as calculated with the formula half – life = ln(2)/k₀, where the half-life represents the time needed for an initial extractible ME concentration to be divided by 2.

The growth data were modelled using a restricted growth model, following equation 2:

$$W(t) = Wmax - ((Wmax - W0) \times e^{(-k_g \times t)})$$

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where $W(t)$ is the individual fresh mass at time t (in g), W_{\max} is the maximal mass reached (in g), W_0 is the mean mass at time $t=0$ (in g), and k_g is the growth rate constant (in d^{-1}). This modelling step was performed to compare growth between exposure modalities and allowed us to estimate the growth constants (k_g) to be considered in the toxicokinetic modelling.

The ME accumulation kinetics in the snail digestive glands were described using a single-compartment toxicokinetic model, as shown in equation 3:

$$C_{sn}(t) = C_{sn}(0) + \frac{a}{k_2 + k_g} \left(1 - e^{-((k_2 + k_g) \times t)}\right)$$

where $C_{sn}(t)$ is the ME concentration in the snail digestive gland at time t (in $\mu g \cdot g^{-1}$), $C_{sn}(0)$ is the ME concentration in the snail digestive gland at time $t=0$ (in $\mu g \cdot g^{-1}$), a is the uptake rate (in $\mu g_{\text{metal}} \cdot g_{\text{snail}}^{-1} \cdot d^{-1}$), k_2 is the excretion rate constant (in d^{-1}) and k_g is the growth rate constant (in d^{-1}). The growth rate constants k_g extracted from the growth model were used here to consider a potential dilution effect due to the growth of the individuals during the experiment.

To assess the ME bioaccumulations in the different modalities, the steady-state concentrations (C_{ss}) were calculated using equation 4:

$$C_{sn}(SS) = \frac{a}{k_2 + k_g}$$

Growth, extractability and toxicokinetic models were fitted using a nonlinear mixed effect procedure (nlme R package), integrating the exposure modalities as fixed effects and the containers as random effects.

For all the models, significant differences between treatments were then checked using F tests (analyses of variance, one-way ANOVA, $p < 0.05$), followed by Wald t tests for post hoc multiple comparisons after reordering treatments in models using the relevel R function (stats R package).

All the statistical analyses were performed using R Studio (R Core Team, 2022: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL <http://www.rstudio.com/>) with the dplyr package for data manipulation, and the graphics were generated using the ggplot2 package.

III.3. Results

III.3.1. Environmental availability

The CaCl_2 extract concentrations at t_0 and the corresponding extraction yields of the MEs are given in Figure 19 and Table 9. Overall, the increase in the total concentration led to an increase in the exchangeable concentration of the three MEs at t_0 . For As and Cd, the mean extraction yields were very low in the uncontaminated soils (0.1% for As and 5% for Cd) but reached 4 to 7% in As50-As100 and 15 to 20% in Cd10-Cd20. For Hg, the extraction yields were greater in uncontaminated soils than in spiked soils, but they globally increased (from 2 to 5%) between low (Hg2) and high (Hg5) total concentrations. The differences in extraction yields indicate different behaviours of the considered MEs, with Cd being more labile than As and Hg being less labile. Regarding the influence of MP, PS particles modified the environmental availability of As at t_0 , with a 19% increase for PS1 As100 (Figure 19 and Table 9). For Cd and Hg, PS addition did not affect their initial environmental availability in any of the tested modalities.

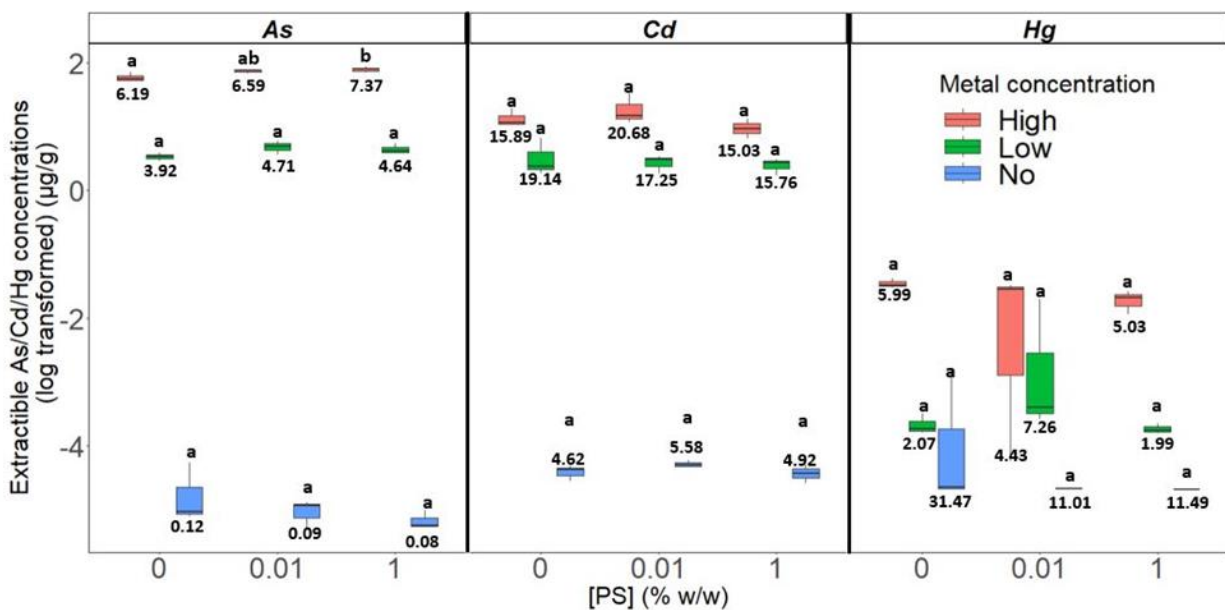


Figure 19: CaCl_2 -extracted concentrations for As, Cd or Hg (in $\mu\text{g}\cdot\text{g}^{-1}$) for each PS concentration (in % w/w) at t_0 . The numbers under the bars correspond to the extraction yields for each modality (in % of the total ME concentration). Different letters (above the bars) indicate significant differences in the extractible concentrations between PS concentrations for each total As, Cd or Hg concentration.

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The temporal patterns of the extractible ME concentrations over the course of the experiment are given in Figure 20. The decrease in the rate constants (k_0) of the MEs extracted by the different modalities, as well as the ME half-lives in the soil, are presented in Table 10. The extraction kinetics for the three MEs followed the same pattern, with a rapid decrease in the ME extract concentrations. For As and Cd, the decrease in ME-extractible concentrations occurred during the first weeks of the experiment, while the Hg-extractible concentrations dramatically decreased to almost 0 during the first days of the experiment (Figure 20). These patterns are described by the decreasing rate constants (k_0), which are clearly ME-dependent; Cd exhibited the lowest values (0.05 to 0.11 d⁻¹) and intermediate values for As (0.11 to 0.15 d⁻¹), while Hg showed the highest values (0.70 to 0.91 d⁻¹). Although not influenced by the total concentration in the case of As and Cd, the decreasing rate constants of Hg reached significant values only for the highest concentration (Hg5) due to its very fast adsorption to soil particles (Table 10).

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Table 9: Initial (at t0) As, Cd and Hg extractible concentrations (in $\mu\text{g}\cdot\text{g}^{-1}$) and extraction yields (in % of the total metal concentration) for the different combinations of ME and PS concentrations. The different letters correspond to significant differences in the concentrations of the extracts between modalities. "ns": non-significant.

PS	0			0.01			1		
	0	50	100	0	50	100	0	50	100
As									
Extractible conc.	0.009 ± 0.004 _a	1.70 ± 0.10 _b	5.88 ± 0.45 _c	0.007 ± 0.001 _a	1.97 ± 0.21 _b	6.46 ± 0.19 _d	0.006 ± 0.0009 _a	1.91 ± 0.16 _b	6.63 ± 0.32 _d
Extraction yield	0.12 ± 0.06 _a	3.92 ± 0.24 _b	6.19 ± 0.47 _c	0.09 ± 0.02 _a	4.71 ± 0.50 _b	6.59 ± 0.19 _{cd}	0.08 ± 0.01 _a	4.64 ± 0.39 _b	7.37 ± 0.35 _d
PS	0			0.01			1		
Cd	0	10	20	0	10	20	0	10	20
Extractible conc.	0.012 ± 0.002 _a	1.68 ± 0.52 _b	3.10 ± 0.46 _c	0.014 ± 0.0008 _a	1.55 ± 0.21 _b	3.61 ± 0.95 _c	0.012 ± 0.002 _a	1.48 ± 0.19 _b	2.67 ± 0.59 _c
Extraction yield	4.62 ± 0.57 _a	19.14 ± 5.91 _b	15.89 ± 2.36 _b	5.58 ± 0.33 _a	17.25 ± 2.35 _b	20.68 ± 5.42 _b	4.92 ± 0.72 _a	15.76 ± 1.99 _b	15.03 ± 9.00 _{ab}
PS	0			0.01			1		
Hg	0	2	5	0	2	5	0	2	5
Extractible conc.	0.03 ± 0.03 _a	0.03 ± 0.004 _a	0.23 ± 0.02 _b	0.009 ± 0.00003 _a	0.08 ± 0.09 _{bc}	0.15 ± 0.12 _c	0.009 ± 0.00006 _a	0.02 ± 0.002 _b	0.18 ± 0.03 _c
Extraction yield	31.47 ± 34.93 _a	2.07 ± 0.34 _{ab}	5.99 ± 0.43 _c	11.01 ± 0.03 _a	7.26 ± 7.84 _{ab}	4.43 ± 3.47 _{ac}	11.49 ± 0.08 _a	1.99 ± 0.16 _b	5.03 ± 0.89 _c

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From the decreasing rate constants, we derived the half-lives of the ME extracts, which indicates the capacity of the MEs to remain easily exchangeable. The results indicate that Hg has the shortest extractable half-life (0-1 day), followed by As (5-8 days), while Cd has the longest half-life (6-14 days) (Table 10). All these results indicate a strong ageing effect, depending on the ME considered but similar, in contaminated soils, regardless of the total concentration. Regarding the influence of PS particles, no effect on the soil capacity to retain As, Cd or Hg was observed.

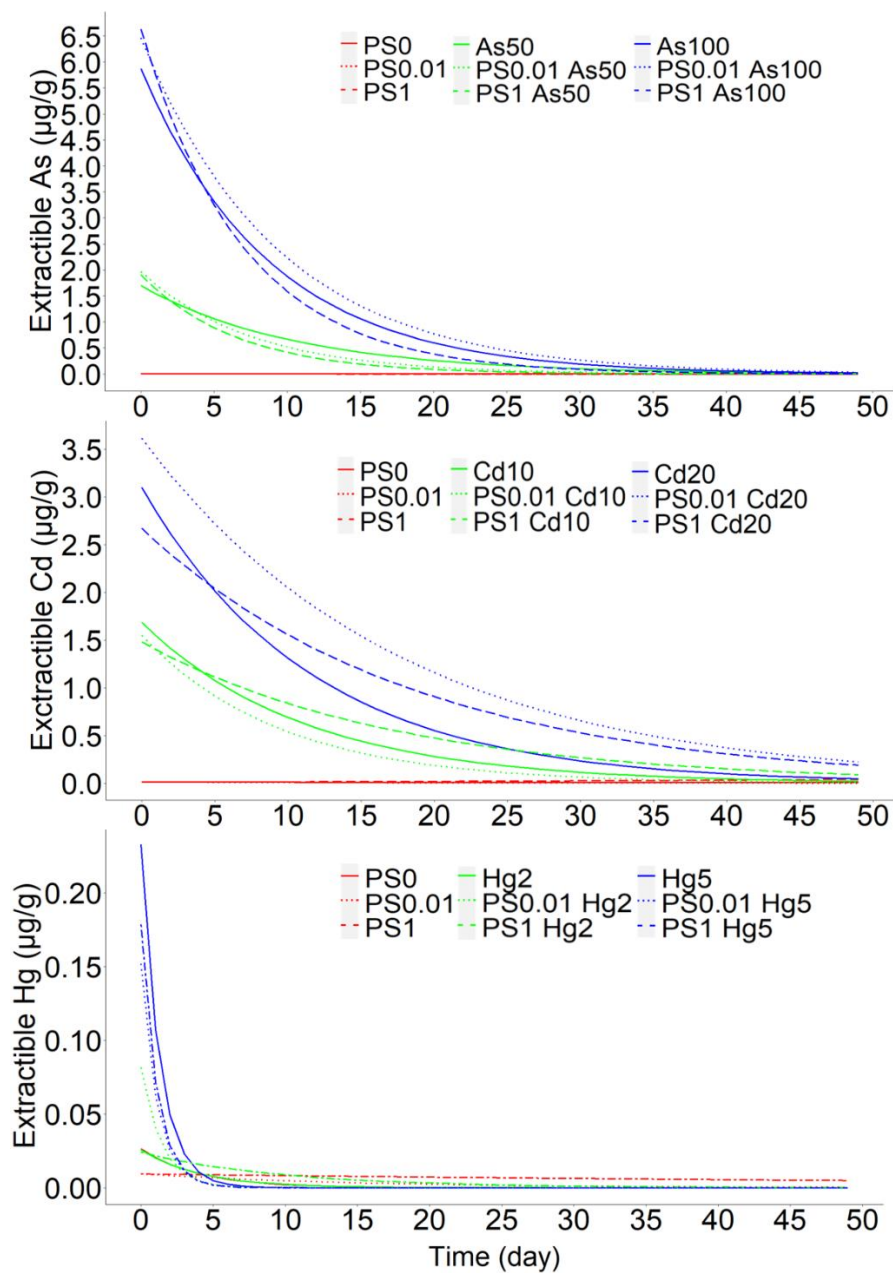


Figure 20: Extraction kinetics of As, Cd and Hg according to the different combinations of ME and PS concentrations.

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Table 10: Decreasing rate constants (k_0 , in d^{-1}) of As, Cd and Hg at different metal and PS concentrations with respect to the metal extractability half-life in the soil for each modality (in d). The different letters correspond to significant differences between modalities. "ns": non-significant. "NC": Not calculated.

PS	0	0.01	1	0	0.01	1	0	0.01	1
As	0			50			100		
k_0	-0.06 ± 0.05 ^{ns}	-0.12 ± 0.05 ^a	-0.23 ± 0.03 ^b	0.09 ± 0.02 ^c	0.13 ± 0.02 ^c	0.15 ± 0.02 ^c	0.11 ± 0.02 ^c	0.11 ± 0.02 ^c	0.14 ± 0.02 ^c
Half-life	NC	NC	NC	7.70	5.33	4.62	6.30	6.30	4.95
PS	0	0.01	1	0	0.01	1	0	0.01	1
Cd	0			10			20		
k_0	0.004 ± 0.03 _{ns}	0.05 ± 0.04 ^{ns}	-0.03 ± 0.03 ^{ns}	0.09 ± 0.03 ^a	0.11 ± 0.03 ^a	0.06 ± 0.02 ^a	0.08 ± 0.03 ^a	0.06 ± 0.02 ^a	0.05 ± 0.02 ^b
Half-life	173.25	13.86	NC	7.70	6.30	11.55	8.66	11.55	13.86
PS	0	0.01	1	0	0.01	1	0	0.01	1
Hg	0			2			5		
k_0	0.25 ± 0.31 ^{ns}	-0.11 ± 0.05 ^{ns}	0.03 ± 0.09 ^{ns}	0.25 ± 0.31 ^{ns}	0.71 ± 0.52 ^{ns}	0.10 ± 0.16 ^{ns}	0.70 ± 0.25 ^a	0.89 ± 0.69 ^a	0.91 ± 0.63 ^a
Half-life	2.77	NC	23.10	2.77	0.98	6.93	0.99	0.78	0.76

III.3.2. Environmental bioavailability

No snail mortality was observed during the 28 days of the experiment. To consider a possible dilution of the internal ME concentrations due to growth, we modelled the ME toxicokinetics by incorporating a growth rate constant (k_g) into the model. The growth during the experiment was homogeneous, and no significant differences were observed between the different modalities, with a mean growth rate constant of $0.056 \pm 0.008 \text{ d}^{-1}$ (Table S.2, in Annex 15). The ME toxicokinetics over the 28 days of exposure to the different soils are presented in Figure 21. The bioaccumulation patterns were similar between the MEs, with a nonlinear shape. These nonlinear kinetics were due to a rapid increase in internal ME concentrations during the first days of the exposure (as described by the uptake rate), followed by a levelling-off of the curve due to elimination processes (growth dilution and excretion), leading to the achievement of a plateau (reflected by the steady-state concentration). These modelled parameters are given in Table 11.

The As and Cd concentrations in lettuce were $0.10 \pm 0.04 \mu\text{g.g}^{-1}$ and $0.72 \pm 0.07 \mu\text{g.g}^{-1}$, respectively. The Hg concentration was below the detection limit. The bioaccumulation of MEs, *i.e.*, the steady-state concentration (C_{ss}), was mostly influenced by the ME exposure concentration. In snails exposed to uncontaminated soil, the C_{ss} was very low, at approximately 1.4, 2.5 and $1.0 \mu\text{g.g}^{-1}$ on average for As, Cd and Hg, respectively. For contaminated soils, the steady-state concentrations increased following an exposure-response relationship, with values reaching approximately 10 to $24 \mu\text{g}_{\text{As}}.\text{g}^{-1}$ for As50-As100, 40 to $67 \mu\text{g}_{\text{Cd}}.\text{g}^{-1}$ for Cd10-Cd20 and 1.9 to $3.5 \mu\text{g}_{\text{Hg}}.\text{g}^{-1}$ for Hg2-g5. These dose-dependent increases in bioaccumulation are related to variations in the uptake rates (a), which increase, in PS-free modalities versus the control, from 1.9 to 4.8-fold for As50 and As100, from 2.4 to 2.9-fold for Cd10 and Cd20 and from 4.3 to 11.0-fold for Hg2 and Hg5. The steady-state concentrations were also dependent on the excretion rates. While very low in uncontaminated modalities, the excretion rates became significant in exposed modalities, *i.e.*, when uptake occurred. In the absence of MP particles in soils, the excretion rates did not significantly vary according to the exposure concentration, maintaining values between 0.11 and 0.27 d^{-1} for As, between 0.07 and 0.09 d^{-1} for Cd and between 0.13 and 0.17 d^{-1} for Hg (Table 11).

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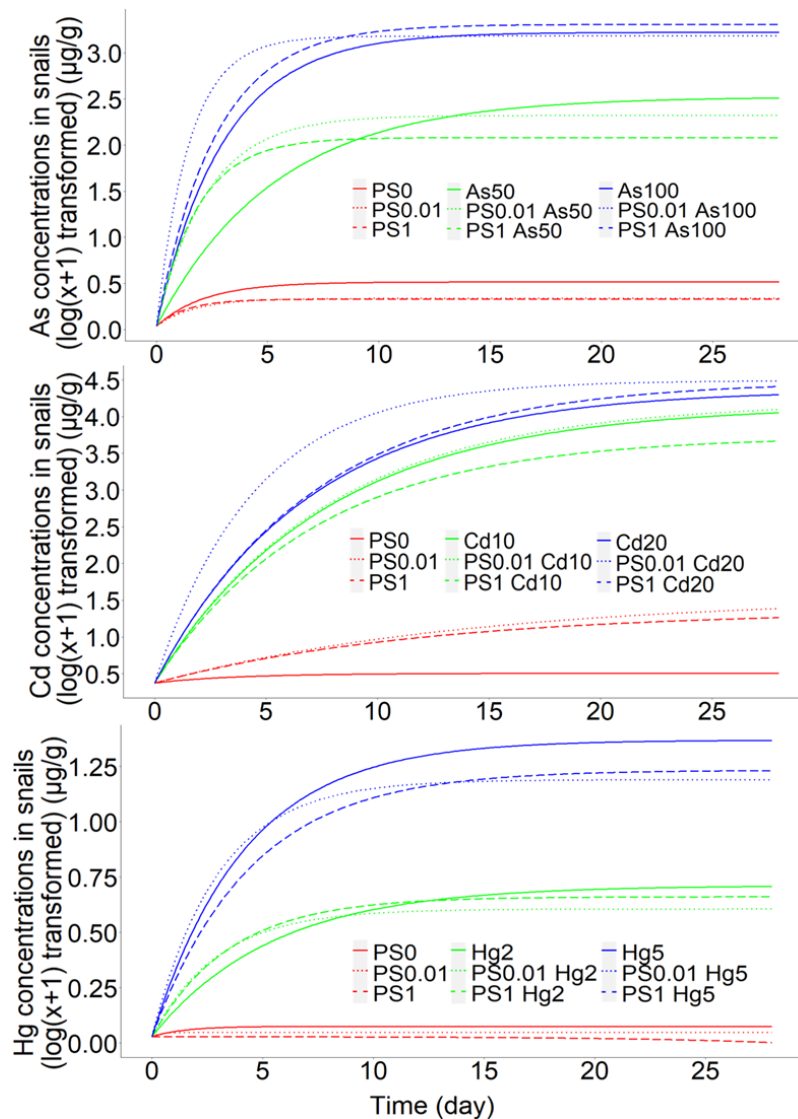


Figure 21: Toxicokinetics of As, Cd and Hg in snails after 28 days of exposure to different combinations of ME and PS.

Co-exposure to PS particles did not lead to important variations in the ME steady-state concentrations (Figure 21). However, their dynamics were modified by the presence of MPs in the soil (Table 11). With respect to As, PS addition increased the uptake rate of As50 by 2- to 3-fold at both PS concentrations. However, for As100, the uptake rate doubled in snails exposed to 0.01% PS only ($2.13 \pm 0.57 \mu\text{g}_{\text{As}} \cdot \text{g}_{\text{snail}} \cdot \text{d}^{-1}$), while no difference was observed for PS1% ($1.28 \pm 0.20 \mu\text{g}_{\text{As}} \cdot \text{g}_{\text{snail}} \cdot \text{d}^{-1}$) compared to the control ($1.05 \pm 0.20 \mu\text{g}_{\text{As}} \cdot \text{g}_{\text{snail}} \cdot \text{d}^{-1}$). Concomitant increases were also observed for the excretion rates. For Cd, whereas no influence of PS was detected at $10 \mu\text{g}_{\text{Cd}}/\text{g}$ soil (Cd10), both the uptake and excretion rates doubled for Cd20 at 0.01% PS ($p=0.01$ and $p<0.001$ for a and k_2 , respectively). However, no change occurred at the highest PS exposure concentration (1%).

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Table 11: Uptake rate (a in $\mu\text{g}_{\text{metal}}\cdot\text{g}_{\text{snail}}^{-1}\cdot\text{d}^{-1}$), excretion rate (k_2 in d^{-1}) and steady-state concentrations (C_{ss} in $\mu\text{g}\cdot\text{g}^{-1}$) of As, Cd or Hg for the different combinations of ME and PS concentrations ($\log(x+1)$ values). The different letters correspond to significant differences between modalities. "ns": non-significant.

PS	0	0.01	1	0	0.01	1	0	0.01	1
As	0			50			100		
a	0.22 ± 0.17 ^{ns}	0.17 ± 0.26 ^{ns}	0.23 ± 0.49 ^{ns}	0.42 ± 0.09 ^a	1.08 ± 0.27 ^b	1.15 ± 0.31 ^{ab}	1.05 ± 0.20 ^b	2.13 ± 0.57 ^c	1.28 ± 0.20 ^{bc}
k₂	0.40 ± 0.43 ^{ns}	0.53 ± 0.99 ^{ns}	0.68 ± 1.35 ^{ns}	0.11 ± 0.05 ^a	0.38 ± 0.12 ^b	0.49 ± 0.21 ^{bc}	0.27 ± 0.07 ^{abc}	0.62 ± 0.19 ^c	0.33 ± 0.09 ^{abc}
C_{ss}	0.50	0.28	0.31	2.37	2.52	2.14	3.10	3.16	3.36
PS	0	0.01	1	0	0.01	1	0	0.01	1
Cd	0			10			20		
a	0.20 ± 0.08 ^a	0.08 ± 0.03 ^a	0.08 ± 0.03 ^a	0.49 ± 0.08 ^b	0.51 ± 0.08 ^b	0.47 ± 0.08 ^b	0.58 ± 0.07 ^b	0.92 ± 0.12 ^c	0.58 ± 0.08 ^b
k₂	0.18 ± 0.12 ^{ns}	0.02 ± 0.05 ^{ns}	0.03 ± 0.06 ^{ns}	0.07 ± 0.03 ^{ab}	0.07 ± 0.03 ^a	0.08 ± 0.03 ^{ab}	0.09 ± 0.03 ^{ab}	0.16 ± 0.03 ^b	0.08 ± 0.02 ^{ab}
C_{ss}	0.89	0.91	0.96	3.77	3.89	3.41	3.92	4.16	4.46
PS	0	0.01	1	0	0.01	1	0	0.01	1
Hg	0			2			5		
a	0.03 ± 0.29 ^{ns}	0.06 ± 0.11 ^{ns}	0.003 ± 0.003 ^{ns}	0.13 ± 0.09 ^a	0.19 ± 0.08 ^a	0.18 ± 0.07 ^a	0.33 ± 0.07 ^b	0.41 ± 0.14 ^{ab}	0.27 ± 0.08 ^{ab}
k₂	0.62 ± 2.58 ^{ns}	3.39 ± 2.58 ^{ns}	0.87 ± 2.58 ^{ns}	0.13 ± 0.07 ^a	0.29 ± 0.17 ^a	0.23 ± 0.12 ^a	0.17 ± 0.07 ^a	0.31 ± 0.14 ^a	0.18 ± 0.08 ^a
C_{ss}	0.05	0.02	0.00	0.75	0.57	0.61	1.49	1.10	1.12

Hence, for As and Cd, the effects of PS on the uptake rates followed non-monotonic patterns, with the lowest PS concentration (0.01%) exhibiting stronger responses than the highest PS concentration. Lastly, for Hg, neither the uptake rates nor the excretion rates were affected by PS particles regardless of their concentration in the soil.

III.4. Discussion

III.4.1. Metal environmental availability is mostly driven by the metal concentration

All the MEs tested in this experiment have been shown to follow relatively rapid adsorption kinetics in soils (Yu et al., 2023). During the ageing process, the environmental availability of the tested MEs rapidly decreased during the first three weeks of equilibration. The CaCl₂-extractable fraction represents the labile, easily exchangeable fraction of MEs in soils that can directly interact with soil components. MEs can be adsorbed onto soil particles through electrostatic interactions, hydrophobic interactions, hydrogen-bonding interactions, π - π interactions or surface complexation (Song et al., 2022). More specifically, humic substances, which can constitute up to 50% of the natural organic matter in soils, contain different oxygen-containing functional groups, making them negatively charged polyelectrolytic and polyfunctional ligands. Thus, humic substances are able to adsorb or complex positively charged MEs strongly via metal bridging, ion exchange and electrostatic interactions (Coles and Yong, 2006; Lu et al., 2022; Misra et al., 1996; Warwick et al., 2005). With time, the MEs were less prone to desorption, and their extractability decreased. The contrasting half-lives we determined illustrate the ME-dependent abilities to fractionate between soil phases, with Hg being rapidly bound, notably to organic matter (Huang et al., 2020), with As progressively being associated with oxides (Fe, Al or Mn hydroxides), carbonates and organic matter (Motuzova et al., 2006), while Cd was loosely bound to the less stable exchangeable, carbonate and hydrous oxide fractions (Kubier et al., 2019). In the soil, MEs may also interact with MPs (and notably PS, which contains styrene groups) through electrostatic bonding or π - π interactions. Water-based adsorption kinetic experiments revealed fast adsorption equilibrium, *e.g.*, 48 hours for Hg on several MPs (Gao et al., 2023), 24 hours on PS (Zhu et al., 2022), 120 hours for As on polyethylene (Tang et al., 2023) or 20 hours for As on PS (Dong et al., 2020). Here, in the presence of PS particles in soils, there was an increase in As availability

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during the first few moments of co-exposure (at the time of spiking), which has not been described in the literature. The increase in As did not come from the leachate from the raw PS particles, because they did not release a significant quantity of As in 0.01 M CaCl₂ ($0.0034 \pm 0.0009 \mu\text{g}_{\text{As}}\cdot\text{g}^{-1}$ PS). The resulting hypothesis is an electronegative repulsion between PS particles and As anions (arsenate), which are both negatively charged (Dong et al., 2020). Such electronegative repulsion led to an increase in the As fraction towards the liquid phase of the soil (*e.g.*, by limiting the adsorption on the soil solid phase), thus increasing the As extractability at the beginning of the experiment. However, this effect was short-lived, and the exchangeable fraction rapidly decreased, as the As ions were associated with the soil hydroxides. Here, a decrease in ME environmental availability was observed independently of the presence of PS in the soils. In the literature, the impact of MP particles on ME behaviour varied among the studies. On the one hand, Li et al. (2023) reported decreased As and Cd concentrations after one month of equilibration, and this phenomenon increased after the addition of 1% or 10% MP (PE or PBAT) to soils. Similar results were observed following the addition of 0.5 to 2% PS to soils and 15 days of equilibration (Zhang et al., 2022). Similarly, Dong et al. (2021) showed decreased As availability in rhizosphere soils after rice growth in a pot experiment, and the effect further increased after the addition of PS or PTFE. On the other hand, Liu et al. (2023) showed increased DTPA-extractable Cd concentrations in soils after MP addition (0.2 to 2% PET, PLA or PES), while Zhao et al. (2023) increased CaCl₂-extractable Cd concentrations in soils after PS or PP addition. Numerous factors (the size of the MP particles used, the nature of the reactive groups present at their surface, their ageing state, and differences in experimental procedures) may explain the variety of responses and sometimes the discrepancies observed in studies focusing on MP-ME co-occurrences. First, the extraction reactants (*i.e.*, CaCl₂ or DTPA) may target different ME-bearing phases in soil, as CaCl₂ allows for the extraction of easily exchangeable ME fractions in soils, while DTPA enables the extraction of carbonate-bound MEs. Thus, different extraction reactants can lead to different results (Jelecevic et al., 2021; Wang et al., 2021). The size of MP particles has also been shown to be an influencing factor, with smaller particles demonstrating higher ME adsorption capacities due to a higher surface-to-volume ratio, which increases the number of potentially available adsorbing sites (Liang et al., 2022; Nguyen et al., 2022; Pinto-Poblete et al., 2022; Premarathna et al., 2023). Microplastic ageing and weathering can also increase MP surface roughness (Vithanage et al., 2021), even during a single-month experiment (Qiu et al., 2022),

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leading to increased specific surface area and/or the addition of reactive surface groups resulting from the addition of organic substances, the breakage of polymer chains, or the formation of reactive byproducts from the degradation of the polymer surface, thereby influencing the adsorption/desorption capacities of MPs in soils (Cao et al., 2021; Li et al., 2023). For example, Mamtimin et al. (2023) observed greater As adsorption capacities of carboxy (COOH)-functionalized PS particles than of amino (NH₂)-functionalized PS particles. Similarly, the behaviour of MPs may be affected by the formation of eco-coronas, which modify the surface interactions between plastic particles and the surrounding medium. An eco-corona is a layered structure that has adsorbed onto an MP surface after environmental exposure; it is derived from abiotic environmental media and bacterial and/or fungal communities present in the soil and is primarily composed of inorganic ions (including MEs) and organic compounds (proteins, lipids, and organic acids) (Cao et al., 2022; Shi et al., 2023). Eco-coronas can form within a few hours through direct sorption to the surface of MPs, subsequently influencing the MP adsorption capacities by changing their surface properties, with MEs being adsorbed both within the corona or on top of it (Yao et al., 2023). These changes can modify the interaction between MPs and MEs and can subsequently modulate their environmental availability as well as their bioavailability, depending on the nature of the molecular compounds forming the eco-corona (Liu et al., 2022).

III.4.2. Subtle effect of microplastics on metal bioavailability

Here, the increased ME concentrations in soils led to an increase in their environmental availability, thus increasing the potential ME quantities available to snails. Snails are exposed to the easily exchangeable ME fraction through a cutaneous route (foot tegument) and, in addition, to the fraction more strongly bound to soil particles through a digestive route (Cœurdassier et al., 2002; Louzon et al., 2020; Pauget et al., 2016). This characteristic was clearly highlighted by the dose-dependent increase in ME bioaccumulation levels, which are partly dependent on ME environmental bioavailability, which is itself driven by environmental availability. In addition to the bioaccumulation process, the toxicokinetic approach used in this study allowed us to determine, through the estimation of uptake and excretion rates, the physiological processes involved. Once released in the gastrointestinal tract following digestive processes, all the tested MEs can be assimilated and can reach the digestive gland, the main site of ME accumulation in gastropod molluscs (Hopkin, 1989). This process has

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already been demonstrated for snails (Gimbert et al., 2006; Mariet et al., 2016; Pauget et al., 2012) and other soil-dwelling invertebrates, such as isopods (Van Straalen et al., 2005) and earthworms (Kilpi-Koski et al., 2019; Spurgeon et al., 2011). In this sense, uptake rates are relevant indicators of environmental bioavailability, and their increase indicates that more MEs are assimilated by snails. Once inside the snail tissues, nonessential elements such as As, Cd and Hg must be detoxified, either by sequestration or excretion. Snails are able to synthesize specific Cd metallothionein isoforms to ensure Cd sequestration in the digestive gland (Höckner et al., 2011; Pedrini-Martha et al., 2017). Since the turnover of metallothioneins is low (Bebianno and Langston, 1998), the excretion of Cd is generally limited. Like Cd, Hg is categorized as a “Class B” ME and has a strong affinity for sulfur ligands (Nieboer and Richardson, 1980). It will then rapidly bind to thiol groups present on proteins, enzymes, etc., where it will be sequestered and slowly excreted. Lastly, As belongs to the intermediate class of MEs and is therefore able to form complexes with different types of biological ligands (metallothionein-like proteins, granules, etc.). In addition, biotransformation (*e.g.*, from arsenate to arsenobetaine) can occur, because it is also considered a detoxification mechanism in soil invertebrates such as earthworms (Langdon et al., 2003). This characteristic explains the excretion rates in our study, with As having higher excretion rates than the other two MEs.

Contrasting trends in the influence of PS can be found in the literature. Some studies have shown that MPs have no impact on ME accumulation. For instance, the presence of PS particles (0.5% w/w in soil, under 2 mm) in soils or biosolids did not affect Cd accumulation in *Enchytraeus crypticus* (Ozturk et al., 2023). In contrast, Xu et al. (2021) reported an increase in the internal As and Cd concentrations in *Eisenia fetida* co-exposed to PS particles (0.1 to 100 µm; 10 and 100 mg.kg⁻¹) as a result of the disturbance of the earthworm immune and metabolic systems. Other studies using *Eisenia fetida* indicated an increase in Cu and Ni bioaccumulation under co-exposure to polyethylene (PE) particles (30 and 100 µm; 0.01 to 1%; Li et al., 2021) and an increase in Cd bioaccumulation when earthworms were exposed to polypropylene (PP) particles (0.03 to 0.9%; < 150 µm; Zhou et al., 2020). More recently, Li et al. (2023) showed that 130 µm PE particles did not change the Cd concentrations in *Eisenia fetida* at low MP concentrations (0.01 and 0.1%) but increased Cd bioaccumulation at 1%. Lastly, Wang et al. (2019) observed that in *Metaphire californica* co-exposed to polyvinyl

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chloride (PVC) particles, a decrease in As tissue concentrations was associated with deleterious effects on earthworm gut bacterial communities. Our results revealed a PS-related increase in the environmental bioavailability of As and Cd, although this increase did not lead to contrasting bioaccumulation levels due to the related induction of concomitant excretion processes. This enhanced environmental bioavailability is unlikely to be directly related to the internalization of ME-loaded PS particles but rather to a modification of the bioaccessibility of MEs in the snail digestive tract. In fact, the size of the PS particles used (*i.e.*, 80 μm) did not allow them to cross the gut epithelium (Stock et al., 2021). However, the presence of PS particles in the soil may influence the processes driving the behaviour of MEs in the gut lumen of snails in different ways. Different hypotheses support the increase in ME bioaccessibility in the snail digestive tract, such as the replacement of eco-coronas in the digestive tract or the effects of the plastisphere or the MP on the intestinal microbiota. More specifically, once in the digestive tract, the eco-coronas can be detached from the MPs and replaced by bio-coronas under the physicochemical conditions of the gut (Cao et al., 2022). These changes can then lead to the release of previously adsorbed MEs, which are then available for assimilation. If large enough, microplastics in soils also allow for the formation of a plastisphere, a microbial community that forms around MPs and is derived from the environment immediately under the influence of plastic around them (Rillig et al., 2024). Like coronas, the composition of the plastisphere microbiome can influence the adsorption processes of MEs (direct ME adsorption on microorganisms or ME adsorption on the surrounding organic compound they synthesized). Plastisphere biological and biomolecular compositions can change over time or under the influence of the characteristics of the surrounding environment (bulk soil vs. gut), which may impact the bioaccessibility and bioaccumulation of MEs (Yang et al., 2022). The increased As and Cd bioavailability in the presence of a low PS concentration (0.01%) can thus be related to changes in the corona and plastisphere composition in the gut environment following soil ingestion by the snails. However, at higher concentrations (1%), the increase in the bioavailability of As and Cd was no longer evident. When the PS concentration increased, the number of particles also increased, as did the number of MP surfaces available for interactions (modifying leachate of additives or particle aggregation). This shift may induce changes in the structure and/or composition of coronas and/or of the plastisphere (Shi et al., 2023) and, in turn, modify the soil physicochemical microenvironment. This trend may ultimately modulate ME behaviour and fate (Yu et al., 2020). These biphasic response patterns

fall under the definition of hormesis (Calabrese and Baldwin, 2002) and may correspond to changes in the soil environment or the associated coronas and/or plastispheres due to increased PS particle concentrations. Our results are therefore consistent with the growing body of evidence highlighting the relevance of hormetic models for characterizing the ecotoxicological risk of MPs (Rix et al., 2022; Sun et al., 2021).

The mechanisms underlying the effects of MPs on ME bioavailability and transfer in soil organisms are complex, and many questions still remain to be answered to address the issue of MP-ME co-contamination in terrestrial environments (An et al., 2023; Tourinho et al., 2023). Furthermore, the effects of concomitant MP and ME exposure on biological targets, as well as their underlying molecular basis, should also be studied (*e.g.*, metabolomic and transcriptomic studies to assess changes in the metabolic pathways of the animals and in their microbiota) Muhammad et al., 2024) in future research.

III.5. Conclusion

This study demonstrated that the environmental availability and bioavailability of ME to *C. aspersus* snails were ME-dependent and primarily driven by both ME and MP exposure concentrations. Although ME bioaccumulation levels were not very sensitive to the presence of PS particles in soil, the toxicokinetic approach used here allowed us to demonstrate that both As and Cd uptake and excretion rates varied according to the MP concentration. We assumed an increase in ME bioaccessibility in the snail digestive tract when exposed to low PS concentrations, although it was not possible to decipher the underlying mechanisms involved. However, regardless of the presence of eco-/biocoronas or the presence of the plastisphere, the effects were no longer visible as the PS concentration increased. Further studies are therefore needed to understand the hormetic responses related to MP exposure and to unravel the dynamic processes driving their interaction with contaminants co-occurring in the soil ecosystem.

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Chapitre IV A. Evaluation de la possibilité de transferts des microplastiques dans les chaînes alimentaires – Du producteur primaire au consommateur primaire

Titre en anglais : Quantitative tracking of nanoplastics along the food chain from lettuce (*Lactuca sativa*) to snails (*Cantareus aspersus*)

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Résumé

Les écosystèmes terrestres sont un réservoir important pour les contaminations plastiques, incluant les nano- et microplastiques (MNPs). A ce jour, peu d'informations sont disponibles concernant les transferts de MNPs dans les chaînes alimentaires, pourtant, des inquiétudes concernant ces voies d'expositions pour les invertébrés et les organismes plus haut dans les chaînes alimentaires ont été soulevées. Notre but était d'examiner et de quantifier le transfert trophique de polystyrène dopé à l'euporium (Eu-PS) dans une chaîne alimentaire terrestre. L'absorption de particules de 100 nm d'Eu-PS depuis de l'eau jusqu'aux racines et aux feuilles d'une plante (laitue, *Lactuca sativa*) et finalement jusqu'à l'escargot (*Cantareus aspersus*) a été évaluée. La laitue a été cultivée dans une solution d'Hoagland dopée à différentes concentrations d'Eu-PS (15, 150, 1500 $\mu\text{g}\cdot\text{L}^{-1}$) durant trois semaines. Puis, les feuilles de laitue ont été utilisées comme source alimentaire pour les escargots durant 19 jours (1g de laitue par jour). Les particules d'Eu-PS se sont accumulées dans les racines de laitue dans tous les traitements, avec uniquement un transfert limité dans les feuilles (quantités quantifiables uniquement à la plus haute concentration d'exposition, facteur de transfert trophique (TTF) <1). De plus, des effets limités ont été observés concernant la biomasse de la laitue suite à l'exposition à l'Eu-PS. Aucun effet des particules n'a été observé sur les escargots, en dehors d'une diminution constante du diamètre coquillère. De manière générale, notre recherche illustre que les petites particules de MNPs peuvent être absorbées par les plantes via leur système racinaire, avant d'être transportées dans les feuilles. Cependant, nos résultats montrent un transfert limité des MNPs dans les tissus des escargots, associé à une excrétion des MNPs dans les fèces des animaux. Ceci fournit une idée des potentielles contaminations des ressources alimentaires humaines. De plus, les MNPs ont causés des effets à la fois chez la laitue et chez les animaux, ce qui indique un potentiel risque pour la santé des consommateurs de la part des MNPs qui peuvent s'accumuler dans les agroécosystèmes.

Abstract

Terrestrial systems are a significant sink for plastic contamination, including nano- and microplastics (NMPs). To date, limited information is available about the transfer of NMPs up the food web via trophic transfer, however, concerns about this exposure pathway for invertebrates and higher-level organisms have been raised. We aim to examine and quantify the trophic transfer of europium doped polystyrene nanoplastics (Eu-PS; NPs) within a terrestrial food chain. The uptake of 100 nm spherical Eu-PS particles from water through the roots of the plants to the leaves and finally to garden snails (*Cantareus aspersus*) was assessed. Lettuce (*Lactuca sativa*) was cultivated in Hoagland solution spiked with different concentrations of Eu-PS (15, 150 and 1500 µg/L) for three weeks. Then, lettuce shoots were used as food for snails for 19 days at a rate of 1 g of shoots per day. The Eu-PS primarily accumulated in the lettuce roots for all treatments, with a limited transfer to the shoots (only quantifiable in the highest treatment; translocation factor: $TF < 1$). No detectable levels of Eu-PS were found in the snails' digestive gland; however, the Eu-PS particles were detected in their feces (trophic transfer factor: $TFF > 1$). Moreover, only limited effects were observed on lettuce biomass by NPs treatments. No effects of the Eu-PS particles on snails were observed, with the exception of a consistent decrease in the shell diameter. Overall, our research illustrates that NPs can be absorbed by plants through their roots, subsequently transported to the shoots. However, our findings show limited transfer of NPs into snail tissues, but direct excretion into their feces. This provides important insight into the potential within the human food basket. Nonetheless, NPs did cause effects on lettuce and snails, indicating a potential health risk to consumers posed by NPs which can accumulate in crops.

IV A.1. Introduction

Terrestrial systems are a significant sink for plastic contamination, including nano- and microplastics (NMPs; Daghighi et al., 2023; de Souza Machado et al., 2018; Kallenbach et al., 2022). Especially agricultural lands accumulate plastics via a range of sources, including the use of agricultural plastic products, the application of biosolids and composts and the use of polymer-based fertilizers and pesticides (Guo et al., 2020; Kumar et al., 2020). It is estimated that 12.5 million tonnes of plastics are annually used in agricultural production (FAO, 2021) with approximately 5,400 to 23,500 tonnes of NMPs released per year by the agricultural and horticulture sectors into the environment (UNEP, 2021). In addition, non-agricultural sources (e.g., landfills and atmospheric deposition) contribute to the release of NMPs into soils (Rocha-Santos et al., 2023; Wan et al., 2022). As a result, levels of contamination are estimated between 13 and 8,885 particles per kilogram in the first 20 cm of agricultural soils (Boughattas et al., 2021; Li et al., 2022; Sa'adu & Farsang, 2023). This raises concerns about the uptake and impact of NMPs on terrestrial organisms, including invertebrates and plants.

NMPs cause a range of effects on plants at environmentally relevant exposure levels, including a reduction in shoot and root length (Zantis et al., 2023). Moreover, plants can take up NMPs through cracks in their roots (Li et al., 2020; Luo et al., 2022) and their leaves (Sun et al., 2021). Once in the plant, they can be transported from the roots to the shoots via the vascular system (Sun et al., 2021; Luo et al., 2022). The uptake and translocation of nanoplastics were observed from lettuce roots to their shoots with a measured translocation factor of ~3% (Luo et al., 2022). In addition, studies have also observed that invertebrates are able to take up microplastics (MPs) when directly exposed (Cui et al., 2022; Rodrigues et al., 2023). For example, *Cantareus aspersus* snails ingested low-density polyethylene (LDPE) particles mixed with their food and were also able to egest fragments up to a size of 1,000 μm (Colpaert et al., 2022). Another exposure pathway for invertebrates and higher-level organisms is the transfer of NMPs up the food web via trophic transfer (Baho et al., 2021), for example from edible crops to other organisms. To date, only limited information is available about this exposure pathway. Concerns about trophic transfer of NMPs in the terrestrial food chain have been raised (Chae & An, 2020; Li et al., 2023a; Wang et al., 2022).

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Given the importance of trophic transfer, there is an urgent need to better estimate this exposure pathway, as it might also have consequences for humans. To our knowledge only three studies have investigated the potential uptake of NMPs into the food chain, with only one that has quantified the amount of nanoplastics (NPs) that was transferred by pyrolysis gas chromatography–mass spectrometry (Li et al., 2023a). In our study, we examine and quantify the trophic transfer of europium-doped polystyrene nanoplastics (Eu-PS) from lettuce (*Lactuca sativa*) to snails (*Cantareus aspersus*) and determine possible adverse effects of Eu-PS exposure. The use of metal-doped nanoplastics is a novel approach to get an accurate indication where and how much Eu-PS are transferred within the food chain. Lettuce is a worldwide cultivated crop and a common plant used for NMPs toxicity testing (Zantis et al., 2023). In addition, the garden snail is an important biological indicator of soil health (de Vaufleury, 2015). Moreover, they serve as an important food source for other animals, such as frogs and birds, but are also suitable for human consumption (Barker & Watts, 2002; Panebianco et al., 2019).

To determine translocation of NMPs in the lettuce and their trophic transfer to snails, we exposed lettuce grown hydroponically to europium-doped polystyrene particles (100 nm). Afterwards, the lettuce shoots were fed to the garden snails to represent a two-level food chain. Levels of europium were then measured in both lettuce and snails to extrapolate the uptake of NPs. The objectives of the study were: i) to quantify the uptake and transfer of NPs from lettuce to snails, and ii) to determine effects of NPs on the growth and behaviour of the snails. The findings of this study will contribute to an improved understanding of the trophic transfer of NMPs through the food chain and possible consequences on higher-levels consumers.

IV A.2. Material and methods

IV A.2.1. Particle characterization

IV A.2.1.1. Eu-PS nanoplastics

Suspensions of spherical europium doped polystyrene (Eu-PS) were purchased from Bangs Laboratories, Inc (Hirschberg an der Bergstrasse, Germany) and stored at a temperature of 4°C. Particles were supplied as 1% (w/v) aqueous suspensions with a nominal size of 100 nm.

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The fluorescence and shape of Eu-PS 100 nm were characterized by transmission electron microscopy (TEM, JEOL JEM-1400 PLUS, JEOL Ltd., Tokyo, Japan). The hydrodynamic diameter of the Eu-PS suspension was determined using a zetasizer Nano-ZS instrument (Malvern, Instruments Ltd., Royston, U.K.). The concentration of europium (Eu) ions was measured using ICP-MS (PerkinElmer® NexION 2000) and the weight and number of particles in the samples could then be calculated.

IV A.2.1.2. Quantification of Eu chelates in Eu-PS suspension and leaching experiment

To quantitatively determine Eu concentrations as a function of their mass, a series of dilutions of the Eu-PS particles were prepared (Annex 16). By measuring the Eu concentration and identifying the linear relationship between Eu and Eu-PS particle mass, we determined the mass and quantity of polystyrene nanoparticles (PS-NPs) present in our samples.

To identify if Eu chelates would leach from the polystyrene nanosphere, the Eu-PS suspension was tested via dialysis tubing. Eu-PS spiked $\frac{1}{4}$ Hoagland solution (0.1% concentration) was added into dialysis tubes (Pur-A-Lyzer™, Labware, Sigma-Aldrich, Netherlands), which were held in 50 mL tubes filled with $\frac{1}{4}$ Hoagland solution. This was done in a triplicate. In addition, negative and positive controls were also performed. Exposure and control vials were kept at the same conditions as those used to culture the plants (see next section). Samples were taken at different timepoints (including 1 h, 2 h, 20 h, 24 h, 48 h and 72 h) and analysed for Eu presence using ICP-MS.

IV A.2.1.3. Preparation test media

The Eu-PS suspension was first sonicated for 5 mins at 60 Hz (USC200T, VWR, Amsterdam, The Netherlands) and then was spiked in $\frac{1}{4}$ Hoagland solution (pH= 6.0 ± 0.2 ; for composition see Table S1, in Annex 17) to reach three different final PS concentrations: 15 $\mu\text{g/L}$; 150 $\mu\text{g/L}$ and 1500 $\mu\text{g/L}$ (low, medium and high, respectively). The 10 mL Eu-PS suspension contained $\leq 0.09\%$ of NaN₃. This suspension was diluted to create exposure concentrations levels in suspension well below concentration at which effects have been observed (Chang & Lamm, 2003). New treatment solutions were made every three days to ensure the freshness of the

spiked Hoagland solution. Control solutions were prepared similarly, but without addition of Eu-PS.

IV A.2.2. Experimental design and exposure

IV A.2.2.1. Plant cultivation and exposure

Lettuce seeds (*Lactuca sativa*) were purchased from Dutch Garden Seeds (Volendam, the Netherlands). The seeds were sterilized using NaClO (0.5% w/v) for 5 mins before being immersed in deionized water and allowed to germinate and grow in Petri dishes containing wet filter papers (10 to 15 seeds per dish) for 72 h. Subsequently, each germinated seedling was individually cultivated hydroponically in 6 mL tubes (VOS Instruments, The Netherlands) filled with $\frac{1}{4}$ Hoagland solution for a duration of three weeks, as described by Dang et al. (2019) and Wu et al. (2021). Then, each pre-grown seedling was selected and exposed to Eu-PS via the roots. The exposure occurred in 22 mL tubes for 21 days, during which each seedling was subjected to a specific concentration (low, medium or high) of Eu-PS spiked Hoagland solution, or to the negative control (only Hoagland solution).

The number of seedlings per treatment was estimated based on Wu et al. (2021), as well as the average food consumption of one snail for a period of three weeks. Therefore, each treatment had 40 seedlings which were grown in three batches, in order to harvest sufficient leaf biomass as food for the garden snails. The exposure medium of all tubes was renewed every three days. Once harvested, the plants were removed from the exposure medium and washed with deionized water for 5 mins, before being weighted (roots and shoots). Roots and aliquots of shoots per each batch and per treatment were kept at -80°C for future analysis, whereas the remaining shoots were kept at 4°C until they were fed to the snails. All experiments were performed in a climate room at $20 \pm 1^{\circ}\text{C}$, a 18 h light cycle and 70% relative humidity (modified from Colpaert et al., 2021; Wu et al., 2021).

IV A.2.2.2. Snail exposure

The feeding experiment was performed for 19 days and was adapted and modified from the experimental protocol described by Wu et al. (2021). In short, sub-adult *Cantareus aspersus* snails (aged three months; shell diameter of 2.53 ± 0.14 cm and weight of 4.94 ± 0.42 g) were obtained from laboratory animal cultures of the University of Bourgogne Franche-Comté

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(UMR CNRS 6249 Chrono- Environnement, France). The animals were acclimated for six days in the laboratory while being fed with vegetal flour ad libitum (for more details see Gomot-de Vaufleury, 2000). Prior to the exposure experiment, the acclimated individuals were not fed for 24 h to empty their gut.

A total of 18 snails were randomly assigned to the four treatments in 600 mL glass beakers (BRAND®, Sigma-Aldrich, Germany) and were divided in six replicates, each consisting of three snails per beaker. The bottom of the beakers was covered with dampened paper to keep a moist environment. To ensure reliable individual identification, each snail was distinctively marked on the shell for a precise tracking during the experiment. The individuals were fed with either unexposed shoots (control) or Eu-PS contaminated shoots. In each beaker, 6 grams of cut shoots (~ 1 g/snail/day) were introduced as food. As the total leaf production of the cultivated lettuce was not sufficient to feed all snails (1 g of lettuce/snail/day), this diet was supplemented with clean lettuce (50% of total diet). This means snails were fed ~ 0,5 g of exposed and clean leaves per snail per day. The individuals were fed every two days with the same amount for a period of 19 days. Produced feces in each beaker were collected, weighed, and stored cumulatively at -80°C to measure the Eu-PS content. At each feeding interval, the unconsumed leaves in each beaker were removed and weighed to calculate the leaf consumption rate. After 19 days of feeding, the snails were not fed for 24 h to deplete Eu-PS shoots from the gut before analysis (for the whole experimental design setup see Figure S1, in Annex 18).

$$\% L_{consumed} = \frac{(WL_{total} - WL_{unconsumed})}{WL_{total}} \times 100$$

where $L_{consumed}$ represents the leaf rate consumption rate, WL_{total} is the total weight of the given leaves ($WL_{total} = 3$ g) and $WL_{unconsumed}$ represents the total weight of unconsumed leaves.

IV A.2.2.3. Sample preparation and particles measurement

Based on Li et al. (2023a), only the digestive gland and feces of the snails were analysed. Samples of lettuce roots and shoots, as well as snail digestive gland and feces, were transferred from the freezer in 15 mL centrifuge tubes (VWR® Ultra-High Performance, Centrifuge tubes). Digestive glands from each beaker were pooled into three replicates (6

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snails of two beakers) to ensure an adequate amount of biomass for analysis. The same was done for the feces samples to allow for comparison with the results of the digestive gland. The empty tubes were firstly weighed and then the wet weight of the samples was measured (OHAUS®, PIONEER™ PA124 – nearest 10⁻⁴ grams). Then, the samples were dried in an oven (VOS Instruments, VOS-12025) at 60°C for 48 h to 72 h (the time of drying depended on the organic matter content of the sample) and weighted.

Samples were digested using acidic solution; 1 mL of an 8:1 HNO₃ : H₂SO₄ mixture (v/v) was added to each sample and placed in a water bath (LabMakelaar Benelux B.V., The Netherlands) at 70° for 48 h to eliminate the organic matter. Then, 9 mL of Milli-Q Water was added to reach a total volume of V_{total}= 10 mL (solution A). The diluted samples were centrifuged for 10 mins at 4200 rpm and, afterwards, 1 mL of the supernatant was sampled and added to a new tube with 1 µL of 100 ppb concentrated Lutetium (Lu) and 8.9 mL of 1% HNO₃ (solution B; V_{total}= 10 mL). The same dilution procedure was repeated but without Lu; 1 mL of the solution B was sampled and added to a new tube containing 9 mL of 1% HNO₃, reaching a total volume of 10 mL of the final solution. The Eu present in the final solutions after two dilutions was then measured using the ICP-MS. After subtracting the blank to the values from the treatment, the mean and standard error of Eu content, the weight of NPs and number of NPs found per sample were calculated.

IV A.2.2.4. Translocation and trophic transfer factors

To investigate the movement of Eu-PS from one part of a plant to another and their transfer through the food chain (lettuce-snail in this case), the translocation and trophic transfer factors were calculated, respectively. The Translocation Factor (TF), is used to measure the ability of a plant to move Eu-PS from the roots to the shoots and leaves. It was calculated as follows:

$$TF = \frac{[Eu - PS]_{shoots}}{[Eu - PS]_{roots}}$$

where [Eu-PS]_{shoots} represents the concentration of Eu-PS in the plant shoot tissues and [Eu-PS]_{roots} represents the concentration of Eu-PS in the plant root tissues.

The Trophic Transfer Factor (TTF) is defined as the ratio of the Eu-PS concentration in the snail digestive gland to the concentration of the Eu-PS in the lettuce shoots (adapted from Wu et

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al., 2021). The Eu-PS content in lettuce shoots was halved for the TTF as the snails were fed with mixture of clean lettuce (50%) and of exposed cultivated lettuce (50%). The TTF is used to evaluate the transfer of nanoplastics from shoots to the snail's digestive gland, and was calculated as follows:

$$TTF = \frac{[Eu - PS]_{snail}}{[Eu - PS]_{shoots}}$$

In addition, we also looked at the movement of Eu-PS from lettuce shoots to the feces of snails.

IV A.2.2.5. Effects measurement on lettuce and snails

Root and shoot wet weight and dry weight were recorded for the lettuce plants. For snails, we determined impacts on morphometric traits (whole body weight and shell diameter of individuals) as well as on their behaviour (behaviour state, feeding rate and feces production). The fresh weight and shell diameter of every individual were measured before the exposure experiment (see Figure S2, in Annex 19 for instructions of diameter measurements) and once every week during the feeding period. To determine whether the consumption of contaminated lettuce had an impact on the behaviour of the snails, the behavioural state score (BSS) system for each snail was assessed. The BSS is recorded in five levels of activities ranging from 0 (for full retraction into its shell) to 4 (fully extended state with active movement, see Wu et al., 2021). This endpoint was recorded consistently once a week (day 7, 14 and 19) before the feeding period. The feeding and excretion of snails were determined by weighing the consumption of leaves and the feces production. At the end of the experiment, the snails were sacrificed after the starving period. The shell was removed, and the digestive gland was dissected and stored at -80°C for further analysis.

IV A.2.2.6. Statistical analyses

Statistical analysis was performed using R studio (Version 2023.03.0+386). Results are expressed as mean \pm standard error of six replicates. The data were screened for normality and homogeneity of variance using Shapiro-Wilk test and Levene's test respectively. For some endpoints, one of these assumptions was violated. In these cases, if p values were close to α , results should be treated with caution; being highlighted in the text.

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For diameter, biomass and BSS measured on the snails, a two-level nested ANOVA was used to test differences between treatment and control snails from different replicates. The nested ANOVA determines this variability between subsample (the beakers) within control or treatment and separates it from the variation between treatment and control snails, which was used to determine statistically significant differences. For the diameter and biomass endpoints, no effect of the beaker was recorded, thus data from individual snails was used (n=18). For BSS, the beaker influenced the BSS of the snails, thus data per beaker was used (n=6). More information about statistical analysis on all endpoints can be found in Table S2 (in Annex 20). A one-way ANOVA ($\alpha=0.05$) was performed to evaluate if results were statistically significant at a 95% confidence level. A Tukey's post hoc test was performed to determine significant effects among treatments. Adjusted p-values are presented in this study.

IV A.3. Results

IV A.3.1. Characterization of Eu-PS particles

The Eu-PS particles were observed to be spherical by using TEM (see Figure S3, in Annex 21). Their ζ -potential was measured to be -38 mV in Hoagland solution. The particle size was uniform and determined by DLS to be on average 102.3 nm (min. 100.9, max. 104.5 nm). The hydrodynamic diameter of the Eu-PS suspension in Hoagland solution was determined to be 102 ± 2 nm. Minimal leaching of Eu from the particles was detected, with a maximum amount of Eu dissolved in Hoagland solution being 0.049 $\mu\text{g/L}$ at the highest concentration (1500 $\mu\text{g/L}$; see Table S3, in Annex 22)

IV A.3.2. Uptake and effects of Eu-PS on lettuce

Uptake of Eu-PS by roots and shoots of lettuce was observed (Figure 22A-D). Eu was detected in roots for all three concentrations. The mass of particles was estimated to be $3.8 \times 10^{-7} \pm 3 \times 10^{-8}$ mg/g DW at 15 $\mu\text{g/L}$, $4.8 \times 10^{-6} \pm 1.3 \times 10^{-7}$ mg/g DW at 150 $\mu\text{g/L}$ and finally $3.2 \times 10^{-5} \pm 3.1 \times 10^{-6}$ mg/g DW at 1,500 $\mu\text{g/L}$ in lettuce roots (Figure 22A). This translates to $6.9 \times 10^5 \pm 5.5 \times 10^4$, $8.7 \times 10^6 \pm 2.4 \times 10^5$ and $5.7 \times 10^7 \pm 5.6 \times 10^6$ NPs particles in roots for the low, medium, and high treatment respectively (Figure 22C). In contrast to the roots, Eu content was only detected for the high concentration in the lettuce shoots. For the 1,500 $\mu\text{g/L}$ treatment, the mass of NPs found in lettuce shoots was $5.2 \times 10^{-7} \pm 1.8 \times 10^{-7}$ mg/g DW (Figure 22B), or 9.5×10^5

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$\pm 3.2 \times 10^5$ NPs particles (Figure 22D). The translocation factor from roots to shoots was determinate for the highest treatment. We determined the translocation between lettuce roots and shoot to be $0.24 \pm 0.14\%$ for the highest exposure concentration (Figure 23). No detectable levels of Eu-PS were found in the shoots for the low and medium concentration.

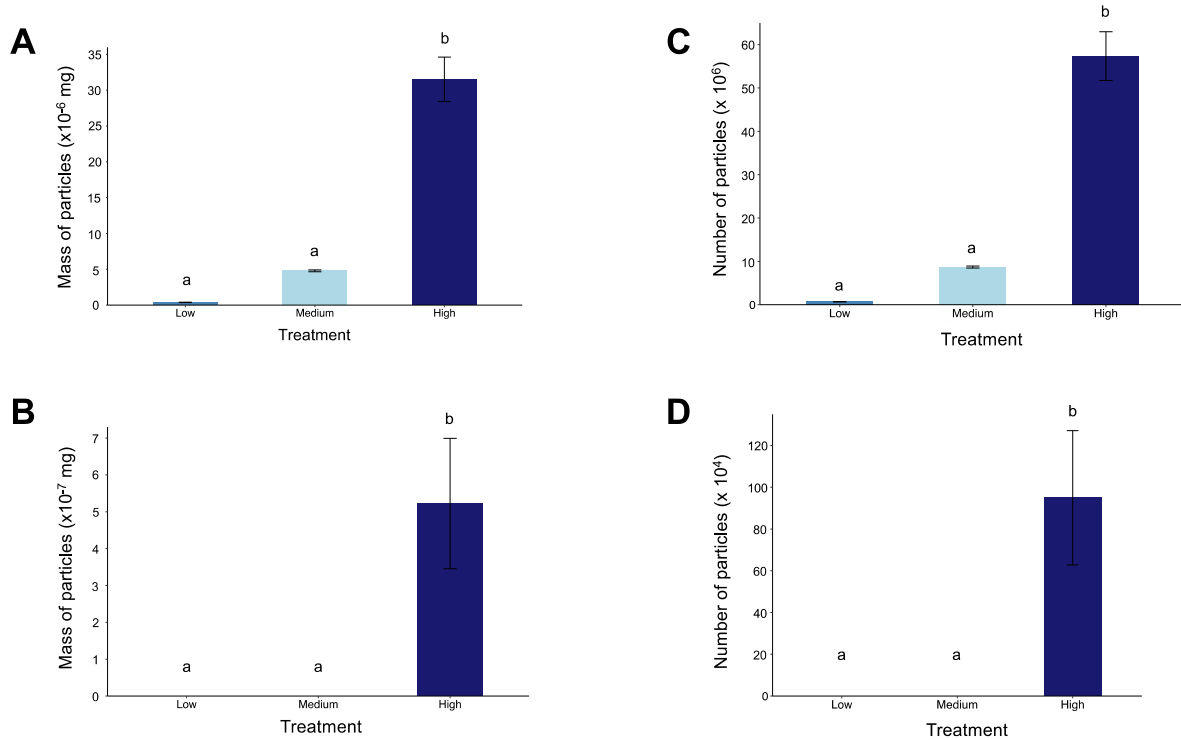


Figure 22: The mean \pm SE weight of particles (mg) relative to the control in roots [A] and shoots [B] and the number of particles in roots [C] and shoots [D] of lettuce (*Lactuca sativa*) after three weeks of exposure to low, medium and high 100 nm Eu-PS concentrations (15, 150, 1,500 $\mu\text{g/L}$). Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p < 0.05$) between treatments. Error bars represent standard errors.

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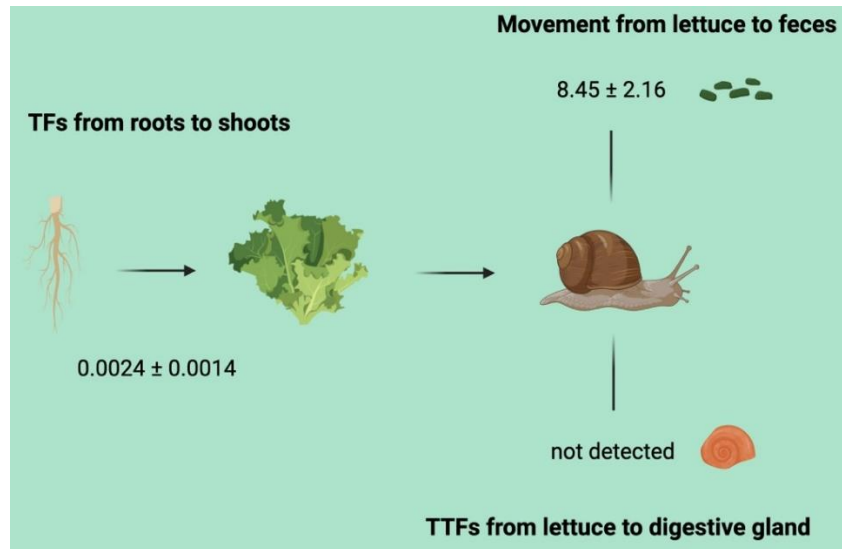


Figure 23: The mean \pm SE translocation factor (TF) of Eu-PS from lettuce roots to shoots after exposure to 1,500 $\mu\text{g/L}$ Eu-PS and trophic transfer factor (TTF) from lettuce shoots to the snail's digestive gland and the movement to feces.

Statistically significant effects on lettuce biomass were found after exposure of lettuce to Eu-PS particles. While no significant effect was recorded on the wet weight relative to control (ANOVA, $p > 0.05$; Figure 24A), an increase in dry weight was recorded for the high treatment relative to control ($p = 0.04$; Figure 24B). The high Eu-PS treatment increased the root dry weight nearly by 65%. No significant effect was recorded on the shoot dry and wet weight exposed to Eu-PS ($p > 0.05$; Figure 24C-D).

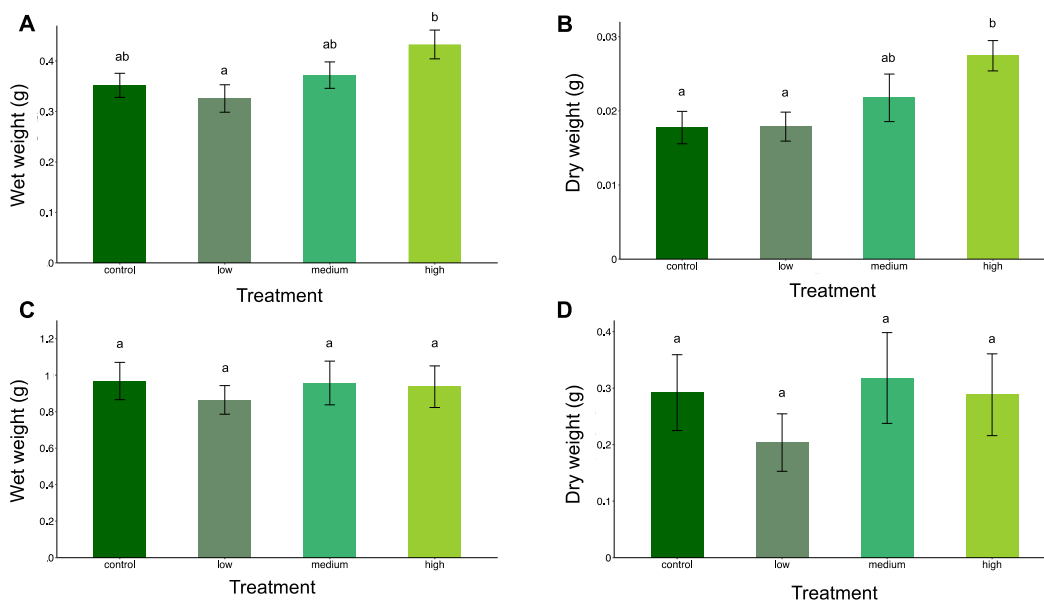


Figure 24: The mean \pm SE root wet [A] and dry [B] weight and shoot wet [C] and dry [D] weight of lettuce (*Lactuca sativa*) after a three week exposure to low, medium and high 100 nm Eu-PS concentrations (15, 150, 1,500 $\mu\text{g/L}$). Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p < 0.05$) between treatments. Error bars represent standard errors.

IV A.3.3. Quantification of Eu-PS transfer from lettuce to snails

Eu content was determined in feces samples at the highest concentration (Figure 25). In feces, the mass of NPs was determined at $2.1 \times 10^{-6} \pm 3.3 \times 10^{-7}$ mg/g DW (Figure 25A), or $3.9 \times 10^6 \pm 6 \times 10^5$ particles (Figure 25B). The movement ratio from lettuce shoots to the feces was higher than one (Figure 23). This means that there was movement of Eu-PS from shoots to the snail's feces. No Eu was detected in the digestive gland (Figure 25). No TTF from shoots to the digestive gland could be calculated as the Eu levels were below detection limit (Figure 23 and 25).

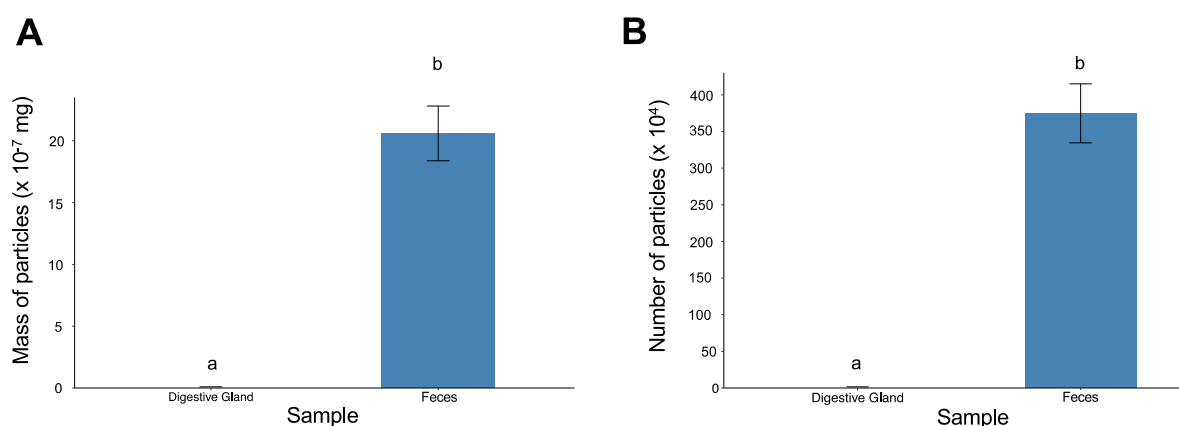


Figure 25: The mean \pm SE weight of particles (mg) [A] and number of particles [B] relative to the control in the digestive gland of snail (*Cantareus aspersus*) and feces samples after exposure to 100 nm Eu-PS contaminated lettuce shoots after exposure to a concentration of 1,500 μ g/L. Levels in digestive gland were below detection limit. Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p < 0.05$) between samples. Error bars represent standard errors.

IV A.3.4. Impact on snail growth and behavior

IV A.3.4.1. Snail growth and behavior

There was no significant effect on the biomass of the snails (ANOVA, $p = 0.19$; Figure 26A). However, the increase in the shell diameter over the duration of the experiment was smaller in the exposed snails compared to control. This was only statistically significant for the snails exposed to the lowest concentration of Eu-PS ($p = 0.037$; Figure 26B). While no significant effect was observed for the medium ($p = 0.18$) and high ($p = 0.06$) concentrations, the increase in shell diameter was still markedly smaller than the controls (Figure 26B). The behavioral activity of

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the snails at three different timepoints was not significantly impacted by the consumption of Eu-PS lettuce ($p>0.05$; Figure 26C).

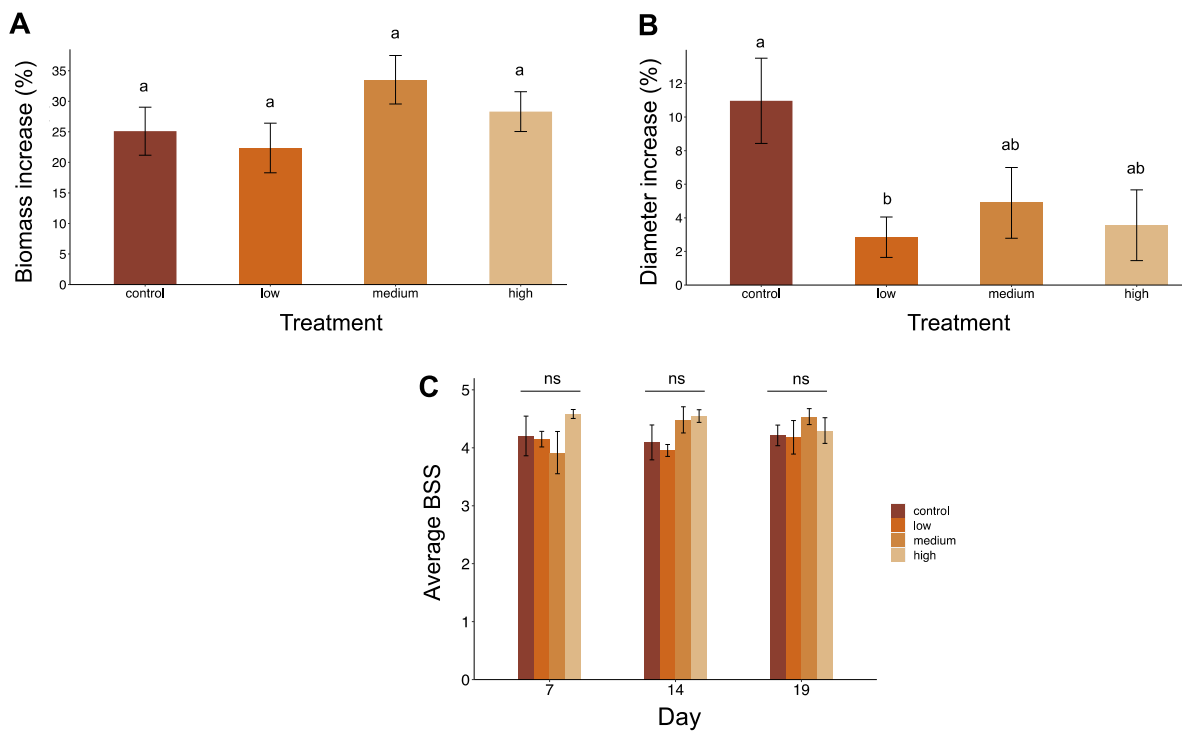


Figure 26: Effect of Eu-PS on the biomass [A] and diameter [B] of snails, and on the average behavioral state score (BSS) [C] upon food chain transfer. Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p<0.05$) between treatments. Error bars represent standard errors. ns: non-significant.

IV A.3.4.2. Snail food intake and feces

At the end of the experiment, no effect was recorded on the total leaf consumption of the snails (ANOVA, $p=0.27$; Figure 27A). In addition, no significant effect was recorded on the feces production of the snails after 19 days (ANOVA, $p=0.4$; Figure 27B).

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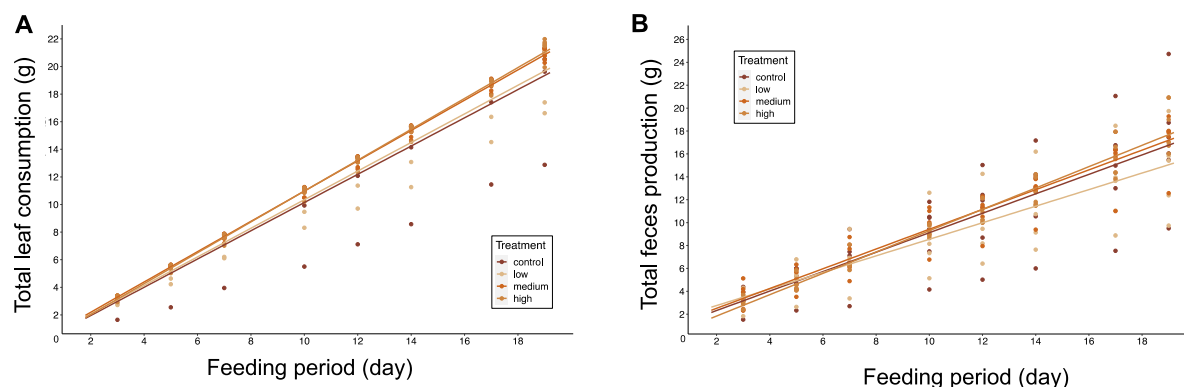


Figure 27: Effect of Eu-PS on the total leaf consumption [A] and total feces production [B] of the snails over time. The lines are fitted through the points as a regression line.

IV A.4. Discussion

This study demonstrated if, how and to what extent metal-doped PS particles are transported from water through lettuce (*Lactuca sativa*) to the terrestrial snail (*Cantareus aspersus*). We determined the fate of Eu-PS within the lettuce-snail trophic chain model, while also assessing their impacts on lettuce growth and snail growth and behavior.

Eu-PS particles were found in both the roots and shoots of lettuce seedlings. This shows that NPs are taken up by the roots and subsequently transferred to the stems and leaves. Our findings are consistent with two other studies that investigated the transfer of NPs from roots to shoots (Li et al., 2023a; Luo et al., 2022). We observed an accumulation of Eu-PS in the lettuce roots of 23, 251 and 1,136 $\mu\text{g/g}$ dry weight at exposure concentrations of 15, 150 and 1,500 $\mu\text{g/L}$. This is in line with another study that found the root accumulation of Eu-PS ranging from 4.1 to 2,220 $\mu\text{g/g}$ dry weight at exposure of 5 and 5,000 $\mu\text{g/L}$, respectively (Luo et al., 2022). The primary routes for NPs to enter plant roots are through the pores in cell walls and the root apical meristem (Li et al., 2020). In addition, NPs can enhance cell permeability by creating new pores, allowing for greater NPs accumulation in root cells (Kurepa et al., 2010). Our study confirms that NPs are accumulating in the roots, and only a small fraction is transferred to the shoots, as the TF from roots to shoots was less than one for the high exposure treatment. Similar to our observed TF (0.24%), Luo et al. (2022) determined the TF to be about 1.8% for 5,000 $\mu\text{g/L}$ (200 nm PS-NPs), while Li et al. (2023a) detected a TF of 2.5% for 1 mg/L, 0.6% for 10 mg/L, 0.3% for 100 mg/L and 0.6% for 1,000 mg/L (50 nm PS-NPs). The

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low transfer rate of NPs from roots to shoots could possibly be explained by the formation of a protein corona (Yu et al., 2023), and that NPs are trapped within certain tissues which hinders the transfer from roots to shoots.

In snails, no particles were detected in the digestive glands. NPs entry into the organism's body is often influenced by the particle type, size, shape and exposure concentration. For example, larger particles tend to persist in the gut, whereas smaller particles possess the capability to traverse cell membranes and to be further translocated to organs (Li et al., 2021). The absence of Eu-PS in our study suggests that NPs were not assimilated in the digestive tract. This would indicate no trophic transfer occurred, which contrasts with the findings reported by Li et al. (2023a), which used higher exposure concentrations.

The TTF values revealed no significant difference among treatment groups with values near zero. The NPs were only detected in the feces of the snails exposed to the highest concentration (1500 µg/L; equivalent to 1.5 mg/L). In a study conducted on the African giant snails, Li et al., (2023a) had similar results to ours for the exposure concentration of 1 mg/L. In that same study, they only noted presence of NPs within the digestive gland and liver at concentrations of 10, 100 and 1000 mg/L, that are substantially higher than ours and considered to be less environmentally relevant. In situations characterized by lower concentrations, the digestive gland has the capacity to detoxify and excrete NPs (Chae & An, 2020; Li et al., 2023a). However, when subjected to elevated concentrations (10, 100, 1000 mg/L), NPs may be accumulated within the digestive gland (Li et al., 2023a). It is important to point out that, in our study, the digestive gland was selected to be analyzed due to its primary role as an accumulation site for NPs to address trophic transfer. Even in the most extreme scenarios involving high concentrations, the vast majority of NPs were excreted in the feces, while a small proportion was found in the digestive gland and less than 1% of the total PS concentration were translocated to the soft tissue of the African giant snails (Li et al., 2023a). This shows that transfer within organs is limited, even at high environmental exposure levels.

Limited effects of Eu-PS particles on both lettuce and snails were detected. Eu-PS particles did significantly impact the biomass of lettuce seedlings; an increase in dry weight for the lettuce roots was observed. On the opposite, a previous study that exposed lettuce to 5,000 µg/L europium doped PS particles showed no impact on plant growth characteristics (Luo et al., 2022). In a recent review, root and shoot biomass on lettuce were commonly assessed, but

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responses vary (Zantis et al., 2023). For snails, the increase in shell diameter over the duration of the experiment was lower compared to control. A reduction in the increase in shell diameter is commonly found as a sensitive endpoint to the exposure to pollutant treatments (Chae & An, 2020; Li et al., 2023a; Wu et al., 2021). For example, snails fed with spiked silver nanoparticles and titanium dioxide nanoparticles lettuce also showed a significant inhibition of the snail diameter (Wu et al., 2021). This suggests that shell diameter is a sensitive endpoint, however it remains unclear how the snails are impacted, as our chemical analysis was below detection limit. One possible explanation is that additives are leaching from the plastics and are interacting with the snail, but more research is needed.

Most studies to date have been done with PS particles (Li et al., 2023a; Chae & An, 2020), but other types of particles, such as polyethylene (PE) and polyvinyl chloride (PVC), are found most in the environment (Sa'adu and Farsang, 2023; Qi et al., 2020). Therefore, there is a need to test the trophic transfer and effects of different polymers as well as mixture of polymers which are commonly found in agricultural soils. Moreover, further research should also focus on the use of aged particles, rather than pristine particles.

Given the significant concern about the presence and quantity of MPs in agricultural soils (Pérez-Reverón et al., 2022; Kumar et al., 2020), it is of crucial importance to understand how MPs move through the food web. This study investigated lettuce and the garden snails (or escargots in restaurants), both of culinary significance. In summary, our research illustrates that NPs can be absorbed by plants through their roots, subsequently transported to the shoots. However, we did not find trophic transfer into snail tissues, but direct excretion into their feces. Our findings reveal a limited transfer of NPs from plants to snails at a higher trophic level, which provides important insight into the potential within the human food basket. NPs did cause effects on lettuce and snails, indicating a potential health risk to consumers posed by NPs which can accumulate in crops.

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Chapitre IV B. Evaluation de la possibilité de transferts des microplastiques dans les chaînes alimentaires – Du consommateur primaire au consommateur secondaire

Titre en anglais : Microplastic trophic transfer in animal tissues along an artificial food chain: From terrestrial snails (*Cantareus aspersus*) to scarabs (*Carabus lefebvreii*)

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Résumé

La contamination de l'environnement par les déchets plastiques est maintenant devenue un problème majeur. Les plastiques sont capables de se fragmenter en fragments de 0,1 à 5 mm, appelés microplastiques (MPs). Les MPs sont plus à risques que les plastiques, leur petite taille facilitant leur rétention dans les compartiments environnementaux et leur transfert dans les organismes. Par conséquent, de telles particules posent un risque de contamination des chaînes alimentaires, incluant plantes, animaux et humains. Malgré l'accroissement du corpus de preuves montrant les effets des MPs, peu d'études se sont intéressées à leur potentiel de transfert dans les chaînes alimentaires, le peu d'études s'y intéressant portant majoritairement sur les écosystèmes aquatiques. De plus, ces études ont essentiellement cherché des MNPs dans le tractus gastrointestinal (TGI) des animaux, laissant un flou sur leur internalisation. Le but de notre étude a été d'étudier le transfert de MPs dans une chaîne alimentaire comprenant deux invertébrés du sol, un consommateur primaire et son prédateur. Pour cela, des escargots (*Cantareus aspersus*) ont été nourris avec de la nourriture contaminée en polystyrène (PS ; 0,01 et 0,1 %), avant d'être utilisés pour nourrir des larves de scarabée (*Carabus lefebvrei*) durant 2 de leurs 3 phases de développement larvaire. Le transfert de PS a été mesuré après retrait du TGI des larves de scarabée grâce à une méthode de détection indirecte utilisant des particules de PS dopées à l'euporium. De plus, les paramètres physiologiques des larves, tel que la survie, le développement larvaire et l'activité phenoloxidase (PO) ont été quantifiés. Les résultats montrent une augmentation des contaminations en MPs à tous les niveaux de la chaîne alimentaire avec une augmentation des concentrations d'exposition (29 à 42 % de transfert de la nourriture à l'escargot, 0,4 à 0,5 de transfert de l'escargot aux larves). Aucune différence de contamination ne fût observée entre les différents stades larvaires, mais les exuvies du second au troisième stade larvaire étaient plus contaminées que les larves, montrant un potentiel mécanisme de détoxification. De plus, même si l'exposition au PS n'a pas entraîné d'effet sur la survie, elle a provoqué une réduction du taux de mue des larves contaminées, et a entraîné une augmentation de leur activité PO durant le troisième stade larvaire. Nos résultats démontrent pour la première fois un transfert de MPs dans une chaîne alimentaire terrestre contaminée. Ce transfert trophique a entraîné une internalisation des particules dans les tissus des prédateurs contaminés, et un effet dose-dépendant sur leur activité PO et leur taux de mue.

Abstract

Plastic littering in the environment has now become a major issue worldwide. Plastics are able to fragment into particles between 0.1 μm and 5 mm in size, so called microplastics (MPs). MPs are of concern, since their small size can facilitate their retention in environmental compartments and their transfer in organisms. Subsequently, such particles pose a risk of contamination for the whole food chains, including plants, animals, and humans. Despite the growing body of evidence on the deleterious MP effects, a few studies, mainly focused on aquatic ecosystems, have already addressed their potential to transfer along the food chains. Furthermore, most of these studies aimed to assess the MP presence in the gastrointestinal (GI) tract of the animals, thus leaving a gap concerning the potential tissular assimilation of the MPs. Our aim was to assess the transfer of MPs in a food chain comprising two soil invertebrates that belong to two different trophic levels, a primary consumer and one of its predators. To do so, *Cantareus aspersus* snails were fed with polystyrene (PS) contaminated food (at 0.01 and 0.1 %) and then used to feed *Carabus lefebvrei* carabid beetles during 2 out of 3 of their larval development. The PS transfer was measured in the carabid larvae after GI tract removal thanks to an innovative indirect quantification method using europium labelled-PS. In addition, physiological parameters, such as survival, larval development and phenoloxidase (PO) activity were quantified. The results showed an increased MP contamination at all levels of the food chain with the increase in contamination, with 29 to 42 % of the particles transferred from the food to the snails, and a 0.4 to 0.5 % of the MPs transferring from the snail to the larva. No different MP concentrations were observed between the different larval stages, but the second to third larval stage exuviae were more contaminated than the larva, highlighting a potential MP detoxification mechanism. Furthermore, even if no significant effect on survival was observed, the exposure produced a significant reduction in moult rate and triggered an increased PO activity in the contaminated carabid beetles during their third larval stage. Our results demonstrate for the first time MP transfer through terrestrial food web contamination. This trophic transfer led to MP internalization in the tissues of the contaminated carabid predator and to a dose-dependent effect on their PO activity and their moulting rates.

IV B.1. Introduction

From the early 50's until nowadays, annual plastic production ranged from 2 million to 391 nearly million metric tons (Plastics Europe, 2022; Zhang et al., 2020). Such increase is explained by their features, *e.g.* being both lightweight and low-cost to produce, as well as being both versatile and durable (Geyer et al., 2017). Approximately 21 % of plastic wastes are recycled or incinerated, the rest being either dumped, landfilled or littered, and thus present a risk of contamination towards the environment (Helm et al., 2023; UNEP, 2021). Even though plastic contaminations were historically mainly studied in aquatic ecosystems (Akdogan and Guven, 2019), plastics are essentially produced and used in terrestrial environment, so it is assumed that terrestrial environments are 4 to 23 times more contaminated than their aquatic counterparts (Horton et al., 2017). Plastic wastes can be categorized according to their size. Microplastics (MPs) are fragments smaller than 5 mm (Van Cauwenberghe et al., 2015). Their lower size limit is still under debate (Frias and Nash, 2019; Abbas et al., 2020) but was set at 0.1µm by the European Food Safety Authority (EFSA) and the International Standardization Organization (European Commission, 2011; ISO 80004, 2015). The terrestrial ecosystems are particularly vulnerable to MPs contamination mainly through agricultural films and organic fertilizers (sludge, compost, digestates) (Fan et al., 2023). Less visible than large debris, MPs are of concern, since their small size can facilitate their retention in environmental compartments and their transfer in organisms. Furthermore, MPs can act as vectors for other pollutants, such as metal(loid)s, organic pollutants or pathogen microorganisms, posing another risk on the biota (Kinigopoulou et al., 2022; Loiseau and Sorci, 2022). Subsequently, such particles pose a contamination risk for the whole food chains, including for humans, as many food items were shown to be contaminated by MPs (Carbery et al., 2018; Huerta Lwanga et al., 2017; Oliveira et al., 2019; Oliveri Conti et al., 2020; Revel et al., 2018; Smith et al., 2018; Wang et al., 2022). Currently, many researches addressing food web contaminations are focused on aquatic ecosystems, both in the wild or under controlled conditions (Carbery et al., 2018; Chae et al., 2018; Da Costa Araújo et al., 2020; Elizalde-Velázquez et al., 2020; Farrell and Nelson, 2013; Lyu et al., 2020; Nelms et al., 2018). From now, only a few studies addressed the MP contaminations and transfer potential in terrestrial ecosystems (Baho et al., 2021a), with for example, plants to snail transfer (Chae and An, 2020; Li et al., 2023), lettuce to flies to fishes transfer (Abdolahpur Monikh et al., 2022), or soil to earthworm to chicken transfer

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In order to fill the gap concerning the MP transfer in soil biota and contamination along the terrestrial food chains, we aimed in this study to assess the potential of polystyrene (PS) MP to transfer along a two levels food chain composed of a primary consumer and a predator, in laboratory conditions. In this purpose, we contaminated with PS particles a vegetal flour (to mimic a contaminated primary producer) to feed *Cantareus aspersus* (Müller, 1774) snails (chosen as a primary consumer, *i.e.* the prey) which were further used to feed larvae of *Carabus (Chaetocarabus) lefebvrei* (Dejean, 1826) (Coleoptera, Carabidae) chosen as a secondary consumer (*i.e.* the predator). We chose these two species because *C. aspersus* is a relevant bioindicator of soil quality owing to its ecological position and roles at the soil-plant-atmosphere interface, and its worldwide distribution in a wide range of habitat (Barker, 2001; de Vaufleury, 2015). Furthermore, *C. aspersus* is a prey for many predators, vertebrates on invertebrates (Barker, 2004). The predator *C. lefebvrei* was selected as this is a relevant soil invertebrate to assess the potential toxic effects of several xenobiotics (Giglio et al., 2021; Talarico et al., 2014) and is known to have a helicophagous behaviour. Polystyrene was used it and can heavily contaminate the agroecosystems (Baho et al., 2021b; Ng et al., 2018), and can also exert deleterious effects on terrestrial biota (oxidative stress, histopathological changes, DNA damage, neurotoxicity or immune and metabolic deregulation; (Qiao et al., 2022). The objectives were 1) to quantify PS particle transfer along the food chain, 2) to determine the potential biomagnification of PS between trophic levels, 3) to assess the impacts of PS particles by monitoring several physiological markers in *C. lefebvrei* and 4) to evaluate the effect of PS

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exposure concentrations on both transfers and effects. We thus investigated the PS concentrations in the snail food and the animals, using indirect measurement methods, to assess the potential contamination through the food web, and the effect of these contaminations on the survival and development of the carabids, as well as the phenoloxidase (PO) activity, which is a key component of the invertebrate immune system (González-Santoyo and Córdoba-Aguilar, 2012), and can be used as an indicator of immunocompetence of insects (Giglio and Giulianini, 2013), in order to appreciate the PS effects on the immunity status of the animals.

IV B.2. Material and methods

IV B.2.1. Plastic particles

The particles used in the experiment were Europium-labelled polystyrene (PS) microsphere (Fluoresbrite® Europium (Eu) Carboxylate Microspheres; Polysciences Europe GmbH, Badener Str.13, 69493 Hirschberg an der Bergstrasse, Germany; abbreviated Eu-PS) with a 0.1 µm mean diameter. The appropriate quantities of particles were suspended in demineralized water and thoroughly mixed in the plant-based flour for snails (Marion®; SAS aliment Marion, 17 impasse du Champs Gaillard, 01290 Saint Jean sur Veyle) to reach 0 (Control), 0.01 and 0.1 % (w/w). The 0.01 % PS concentration corresponds to the order of magnitude (mg/kg) of MPs retrieved in European amended soils (Büks and Kaupenjohann, 2020) and the 0.1 % concentration correspond to a 10-times higher concentration.

IV B.2.2. Animal rearing

Cantareus aspersus snails were obtained from the breeding maintained at Chrono-environnement laboratory (Besançon, France). Adult reproducers have been reared in borosilicate glass boxes under controlled conditions (average temperature of $20 \pm 1^\circ\text{C}$, 70% relative humidity and 18/6 hours light/dark period; Gomot-De Vaufleury, 2000) to produce eggs. After laying, eggs were incubated on a damp paper in glass Petri dishes until they hatched.

Females (n=12) and males (n=8) of *C. lefebvrei* were collected by hand under rotten pine barks in the Catena Costiera Mountain (39°19' N, 16°7' E, 1000 m a.s.l.; Southern Italy, Calabria) in spring 2023. In the laboratory, the beetles were sexed and housed in 10 L boxes (The ratio of

PARTIE B – EVALUATION DE LA POSSIBILITE DE TRANSFERTS DE MICROPLASTIQUES DANS LES CHAINES ALIMENTAIRES–DU CONSOMMATEUR PRIMAIRE AU CONSOMMATEUR SECONDAIRE males to females was maintained at a minimum of 1:1 per box). The boxes were filled with moistened humus to a depth of 6.0 cm to create conditions similar to those in the natural environment, favourable for mating and oviposition. Pine bark and small pieces of rotten wood were used as shelter for the beetles. The beetles were reared at 60% relative humidity with a natural photoperiod and room temperature (day/night: 23/18 °C). They were fed *ad libitum* with snails and *Tenebrio molitor* larvae from a laboratory population maintained at Morphofunctional Entomology Laboratory, University of Calabria. Egg laying was recorded weekly. Eggs were individually transferred to a 30 mL paper cup filled with 1.0 cm depth moistened bark shavings until hatching.

IV B.2.3. Experimental setup

IV B.2.3.1. Eu-PS transfer from food to snail

Newly hatched *C. aspersus* snails were exposed in 2L borosilicate glass containers, the bottom of which has been covered with dampened paper, under controlled conditions ($20 \pm 1^\circ\text{C}$, 70% relative humidity and 18/6 hours light/dark period). Snails (batches of 100 random individuals,) were fed *ad libitum* with 0, 0.01 or 0.1 % Eu-PS contaminated snail flour provided in glass Petri dishes. Snails were fed during 7 or 14 days to provide preys appropriately sized for first (non-exposed) and second/third stage (control and exposed) carabids, respectively (see below). In each exposure modality, flour and snail samples were collected and frozen for further Eu analysis. To quantify carabid larvae exposure (as they consume their prey entirely), the whole soft tissues of snails were considered (*i.e.*, without the shell but including the digestive tract).

IV B.2.3.2. Eu-PS transfer from snails to carabid larvae

The experimental design of the larvae exposure and analyses is resumed in figure 28. The larvae were exposed individually in 30 mL paper cups filled with 1.0 cm depth moistened bark shavings (single replicates; natural photoperiod and room temperature). Newly hatched *C. lefebvrei* larvae were fed snails every 48 hours until 3rd stage. It was ensured that each larva was provided of the same number of snails of equal size. In order to ensure consistency in experimental conditions, all early developmental stages were fed with uncontaminated snails. Some specimens were at the egg stage, while others had already hatched when they were

PARTIE B – EVALUATION DE LA POSSIBILITE DE TRANSFERTS DE MICROPLASTIQUES DANS LES CHAINES ALIMENTAIRES–DU CONSOMMATEUR PRIMAIRE AU CONSOMMATEUR SECONDAIRE placed individually in the cups. In order to avoid differential assessment of the duration of the first larval stage, the larvae were all fed with uncontaminated snails until the first moult. The first larval stage was not exposed (as they can use remaining embryonic reserves) and fed with non-contaminated 7-day-old snails (weighting about 35 mg). The exposure started just after the first moult. Second stage larvae were then randomly divided into three groups (control: 87, 0.01%: 88 and 0.1%: 86), fed with corresponding 14-day-old snails (weighting about 150 mg), and the diet was maintained until the end of the experiment. The experiment was repeated twice.

Larvae (n= 12) were sampled seven days after the first and second moult, which corresponded to the second and third larval stages, respectively, in control and Eu-PS exposed modalities. The larvae were then cold anesthetized (4°C) and dissected to remove the gut. This was done to detect PS-Eu particles that were truly transferred into the tissues and exclude those present in the digestive tract. The samples were stored at -20°C until chemical analysis.

The exuviae were collected after the second larval moult (transition between 2nd and 3rd larval stage) and stored in a sterile tube until chemical analysis. This was done to determine if any particles could be shed during the transition to the next larval stage.

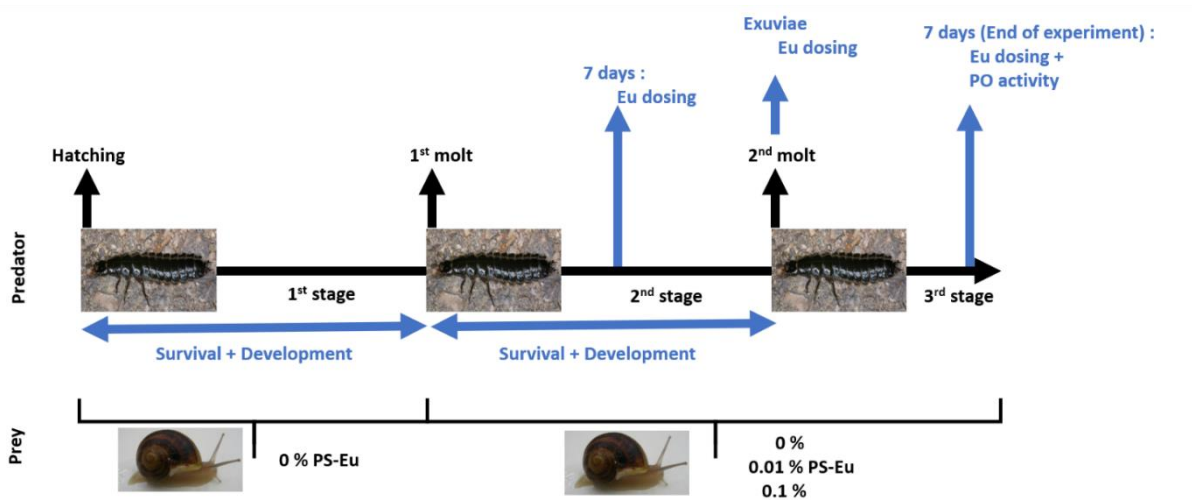


Figure 28: Experimental design of the *Carabus lefebvrei* larvae exposure and analyses strategy. The black arrows correspond to the timeline of the exposure. The blue arrows correspond to the analysis strategy.

IV B.2.4. Europium quantification

IV B.2.4.1. Chemical analyses and transfer factor

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Freeze-dried flour, snail and carabid larvae tissues and exuviae were digested in 1.75 mL HNO₃ (Optima grade, Sigma-Aldrich Chimie SARL) in a Digiprep™ mineralizing apparatus (SCP Science, Quebec, Canada). The volumes were then adjusted to 12.5 mL ultrapure water and Eu dosed by ICP-MS.

The trophic transfer factor (TTF) is the ratio of the Eu-PS concentration in the tissues of an animal to the concentration of Eu-PS in its food source, and his defined by the equation 1:

$$TTF = \frac{[Eu - PS]_{Consumer}}{[Eu - PS]_{Food}}$$

When a transfer of MPs or other contaminant occurs in a food chain, a TTF above 1 indicates a MP biomagnification across the two levels of the food chain, while a TTF under 1 shows no biomagnification across the two levels of the food chain (Mathews and Fisher, 2008).

Here, the TTF in the snail corresponds to a “Trophic Uptake Factor”, as the animals were analyzed as a whole comprising the GI tract (which is considered an external media). The Trophic Uptake Factor then corresponds to the fraction of MPs trophically available for the predators from an upper level. The TTF in the carabid beetles correspond, in this experiment, to a “Truly Trophically Assimilated Factor”, as only the soft tissues of the animals were analyzed, with the exclusion of the GI tract. The Truly Trophically Assimilated Factor thus correspond to the fraction of MPs effectively internalized and transferred to the tissues of the animal.

IV B.2.4.2. Quantitative estimations of the particle load in samples

According to the Eu-PS retailer, the Eu-PS stock solution contains 1.8.10¹⁰ particle per µL stock which corresponds to 0.135 µg Eu. This Eu concentration allowed us to calculate the number of PS particles present in the samples. Considering a density of 1.06 g.cm⁻³, it was also possible to determine the PS concentration in the different matrices. The values of the calculated number of particles and the equivalent PS masses were expressed after subtraction of the background noise, corresponding to the Eu values of the controls.

IV B.2.5. Survival and development time

The survival and development time of the larvae were recorded every 48 hours from hatching to the end of 2nd stage. To evaluate the impact of exposure in PS-Eu groups (0.01% and 0.1%)

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IV B.2.6. Immune responses

To evaluate the effect of exposure to Eu-PS on immune responses of larvae, the enzymatic activity of plasmatic phenoloxidase was measured spectrophotometrically as dopachrome formation from 3,4-dihydroxy-L-phenylalanine (L-DOPA) as in Naccarato et al., 2023. Briefly, the haemolymph was collected from the 3rd stage larvae 7 days after the second moult for each experimental group (0.01% and 0.1% EU-PS treated and control groups). The soft cuticle between the second and third dorsal abdominal segment was pricked with a 26-gauge needle at the level of the hemolymphatic vessel and the first droplet of 10 μ L of haemolymph was collected. A pool of 20 μ L of haemolymph was collected from two specimens, promptly diluted 1:5 into in cold sterile phosphate buffer (PBS, 10 mM; Sigma-Aldrich, Milan, Italy) at 4 °C, and centrifuged at 10⁴ rpm for 5 min at 4 °C. The cell-free haemolymph obtained as supernatant was collected and stored at -20 °C until enzymatic assays for control and Eu-PS exposed groups.

To assess total PO, the zymogen was activated by adding 5 μ L of α -chymotrypsin (5mg mL⁻¹ in cold sterile PBS) to 10 μ L of supernatant (cell-free haemolymph–PBS mixture, 1:10 diluted) in a sterilized 96-well microtiter plate. The mixture was then incubated for 5 minutes at room temperature. To measure basal PO, 10 μ L of cell-free haemolymph was added to each well and mixed with 5 μ L of PBS, the vehicle used to dilute the α -chymotrypsin activator. Then, 80 μ L of L-DOPA (3-(3,4-dihydroxyphenyl)-L-alanine; L-3-hydroxytyrosine, Sigma-Aldrich; 3 mg mL⁻¹ in PBS) was added to each well. The blank was loaded with the same volume of PBS in place of the cell-free haemolymph. The absorbance change was recorded at 492 nm and 25°C for 60 minutes at 15-second intervals using a plate reader (Thermo Scientific Multiskan FC). All samples were analysed in duplicate and expressed as the mean of two technical replicates. Enzyme activity was measured as the slope (absorbance vs. time) of the reaction curve during the linear phase of the reaction (V_{max} value, considering 15 minutes after the start of the reaction).

IV B.2.7. Statistical analysis

The europium concentrations used were log-transformed to encounter the normality and homoscedasticity prerequisites of parametric analyses, then differences were checked using F-tests (analyses of variance, one-way ANOVA, $p < 0.05$), followed by Wald post-hoc multiple comparisons after re-ordering treatments in models using the `relevel` R function (`stats` R package) with the exposure concentration for the samples on the same position on the food chain or the position on the food chain for the samples with the same exposure concentration as explanatory variables. As the data for PO activity did not exhibit a normal distribution (Shapiro-Wilk test, $p\text{-value} < 0.05$), a non-parametric method of analysis was employed: Kruskal-Wallis test followed by pairwise comparisons using the Wilcoxon rank-sum test with Benjamin Hocking correction.

Survival and development analysis were performed with a Cox proportional hazards model with replicates as random effect using the `coxme` function from the package “`survival`” (Eaton et al., 2020). Moulting rates were analysed using a binomial generalised linear model (Bates et al., 2014). Differences between experimental conditions were tested using a one-way analysis of variance followed by a Tukey test as a post-hoc multiple comparison test with Benjamini-Hochberg adjusted p -values. The data were previously tested for homogeneity of variance using the Levene test. Survival and development analyses include 'right-censored observations'. This occurs when a larva is followed for a period of time without the event of interest (molt or death) occurring, but the sample was not followed for the entire treatment period as it was removed for dissection and other analyses. In the graphs, the curves show breaks (|), when a censoring event occurs. The results are presented as mean \pm standard error.

All the statistics were done using R Studio V3.0.1 (R Core Team, 2022: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL <http://www.rstudio.com/>), the GLMM package (Bates et al., 2015), and the `coxme` package (Therneau, 2012), with the `dplyr` package for data manipulation (Wickham et al., 2023), the graphics were done using `ggplot2` package (Wickham, 2016).

IV B.3. Results

IV B.3.1. Eu-PS quantification

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The europium concentration evolution along the food chain for each exposure modality is given in Figure 29. The calculated number of particles and the equivalent PS masses are given in Table 12. Firstly, we observed a significant increase in the europium concentrations with an increase in Eu-PS concentrations in the snail food ($p < 0.001$ between all the concentrations), as well as in the snails ($p < 0.001$ for CTL vs PS 0.01 or vs PS 0.1, $p = 0.047$ for PS 0.01 vs PS 0.1) and in the 2nd or the 3rd stage larvae ($p < 0.001$ between all the concentrations) or their moult ($p < 0.001$ between all the concentrations), as shown in Table 12. Here, the background Eu concentrations in the controls ranged from $0.001 \pm 0.0005 \mu\text{g.g}^{-1}$ in the larvae to $0.110 \pm 0.011 \mu\text{g.g}^{-1}$ in the food. In the exposed modalities, the Eu concentration ranged from $0.003 \pm 0.001 \mu\text{g.g}^{-1}$ in the larvae to $1.444 \pm 0.063 \mu\text{g.g}^{-1}$ in the food in the 0.01 % group, and from $0.011 \pm 0.004 \mu\text{g.g}^{-1}$ in the third stage larvae to $10.647 \pm 1.202 \mu\text{g.g}^{-1}$ in the food in the 0.1 % group. Secondly, the Eu concentration decreased alongside the food chain, as shown in figure 29, with a decrease in Eu concentrations from the snail food to the snails by an order of magnitude (from 1.44 ± 0.063 to $0.60 \pm 0.037 \mu\text{g.g}^{-1}$ in the 0.01 % group, and from 10.65 ± 1.20 to $2.95 \pm 2.18 \mu\text{g.g}^{-1}$ in the 0.1 % group, $p < 0.001$ for the snail flour vs snails, for all the concentrations) and from the snails to the second and third larval stages by one to two orders of magnitude ($0.003 \pm 0.001 \mu\text{g.g}^{-1}$ for the 2nd and 3rd larval stages in the 0.01 % group, 0.014 ± 0.004 and $0.011 \pm 0.004 \mu\text{g.g}^{-1}$ in the 2nd and 3rd larval stages in the 0.1 % group, $p < 0.001$ for the snails vs the 2nd or 3rd larval stage, for all the concentrations).

The Eu concentration between the two larval stages did not significantly differed ($p = 0.8$ for the 0.01 % concentration, $p = 0.1$ for the 0.1 % concentration), but interestingly, the Eu concentration in the exuviae were significantly higher than in the body mass by an order of magnitude (16.3-fold enrichment in the exuviae for the 0.01 and 12.2-fold enrichment 0.1 % groups, $p < 0.001$) as shown in Table 12. The TTF, as shown in Table 12, did not showed Eu-PS biomagnification across the levels of the food chain in the experiment, with TTF ranging from 0.004 to 0.423 in the exposed modalities.

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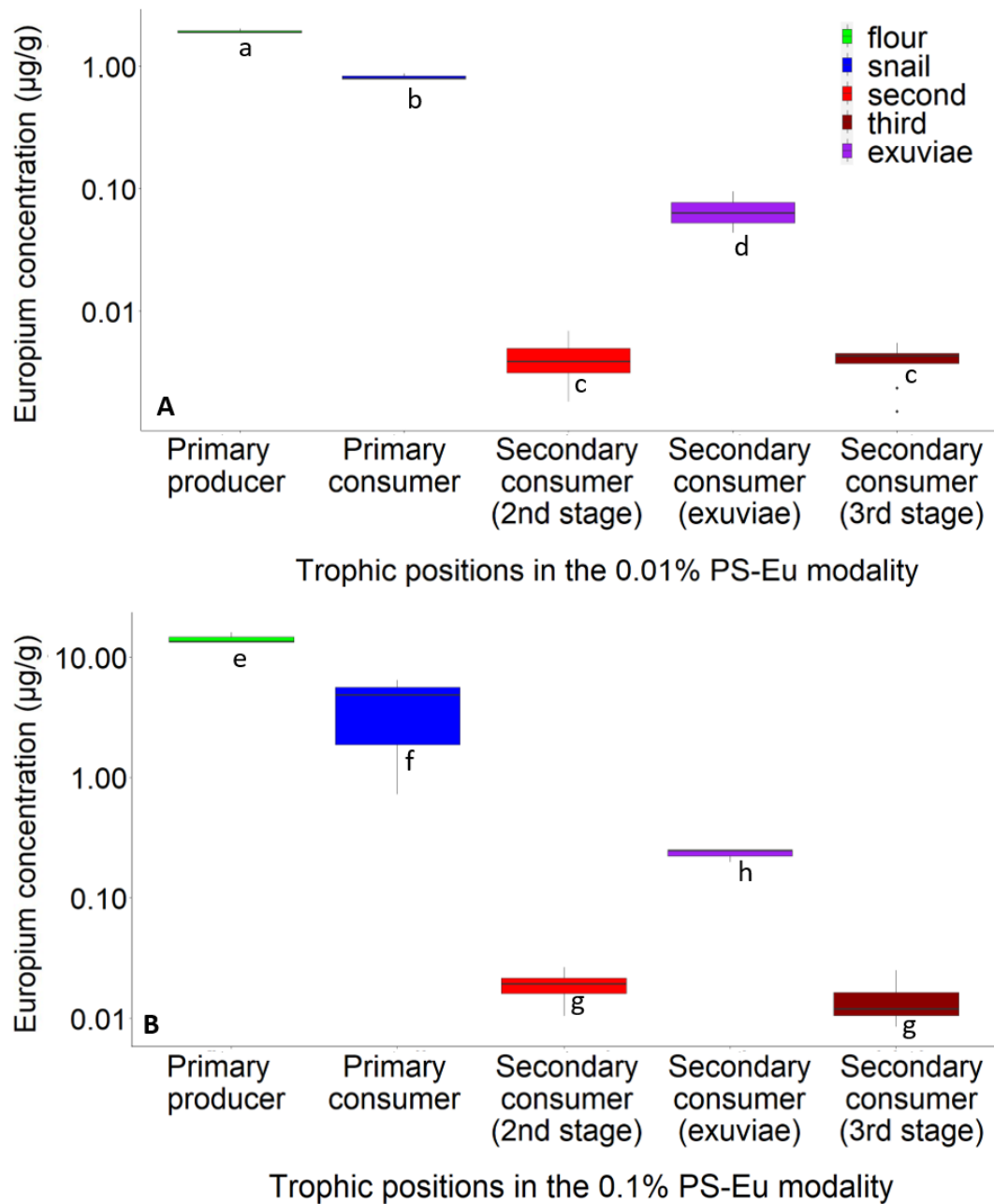


Figure 29: Europium concentrations at the different food web levels for the 0.01 % (Figure 29.A, upper panel) and 0.1 % (Figure 29.B, lower panel), after background Eu concentration from the control being subtracted. The terms “second”, “third” and “exuviae” in legend correspond to the second and third carabid larval stages, as well as the exuviae from the second to third larval stages moulting. The different letters correspond to significant differences between the different trophic levels for each exposure concentration.

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Table 12: Eu concentrations, number of PS particles and PS masses, per gram of biological sample, as well as the trophic transfer factors for the different food web levels for the 0.01 % and 0.1 % PS-Eu contaminated modalities.

Trophic position	Flour	Snail	Second phase larvae	Exuviae	Third phase larvae
MP concentration			0%		
Eu concentration (µg/g)	0.110 ± 0.011	0.045 ± 0.020	0.001 ± 0.0005	0.022 ± 0.002	0.002 ± 0.001
MP concentration			0.01%		
Eu concentration (µg/g)	1.444 ± 0.063 ^a	0.601 ± 0.037 ^b	0.003 ± 0.001 ^c	0.049 ± 0.019 ^d	0.003 ± 0.001 ^c
Particle number.g⁻¹ *	1.78.10 ¹¹ ± 0.84.10 ¹⁰	7.41.10 ¹⁰ ± 0.49.10 ¹⁰	2.19.10 ⁸ ± 1.53.10 ⁸	3.62.10 ⁹ ± 2.52.10 ⁹	1.30.10 ⁸ ± 1.27.10 ⁸
µg PS.g⁻¹ *	98.70 ± 4.68	41.13 ± 2.70	0.122 ± 0.085	2.01 ± 1.40	0.072 ± 0.071
TTF	-	0.417	0.001	-	0.002
MP concentration			0.1 %		
Eu concentration (µg/g)	10.647 ± 1.202 ^e	2.952 ± 2.181 ^f	0.014 ± 0.004 ^g	0.171 ± 0.022 ^h	0.011 ± 0.004 ^g
Particle number.g⁻¹ *	14.05.10 ¹¹ ± 1.60.10 ¹¹	3.88.10 ¹¹ ± 2.91.10 ¹¹	16.85.10 ⁸ ± 4.87.10 ⁸	19.83.10 ⁹ ± 2.99.10 ⁹	12.21.10 ⁸ ± 5.91.10 ⁸
µg PS.g⁻¹ *	779.74 ± 88.98	215.07 ± 161.36	0.935 ± 0.270	11.00 ± 1.66	0.68 ± 0.33
TTF	-	0.276	0.004	-	0.003

IV B.3.2. Survival

No significant mortality of carabid larvae was observed during the experiment, and no differences were observed between the different diet regimes (Figure S1, in annex 23), with

PARTIE B – EVALUATION DE LA POSSIBILITE DE TRANSFERTS DE MICROPLASTIQUES DANS LES CHAINES ALIMENTAIRES–DU CONSOMMATEUR PRIMAIRE AU CONSOMMATEUR SECONDAIRE mortalities of 1.92 ± 2.72 % in the controls, 5.88 ± 2.16 % in the 0.01 % PS-Eu group, and no mortality the 0.1 % PS-Eu group ($p=0.12$).

IV B.3.3. Development time

The non-exposed first larval stages showed no difference ($p=0.99$) concerning their moulting rates (Figure S2, in annex 24), all the individualized larvae reached the second stage. However, once the different dietary treatments were implemented, a considerable variation in the moulting rates of the second larval stages was observed (Figure 30). In the controls, 76.40 ± 11.62 % of the larvae moulted, while the moult rate in the 0.01 % PS-Eu group was lower than the control (moulting rate: 56.04 ± 0.68 %, $p=0.02$). The 0.1 % PS-Eu group did not differ from the other groups ($p= 0.8$ and $p=0.09$ vs CTL or 0.01 %, respectively) with a 66.78 ± 1.98 % moulting rate. Furthermore, no significant differences in the development time ($p=0.47$) was observed between the modalities (Figure S3, in annex 25).

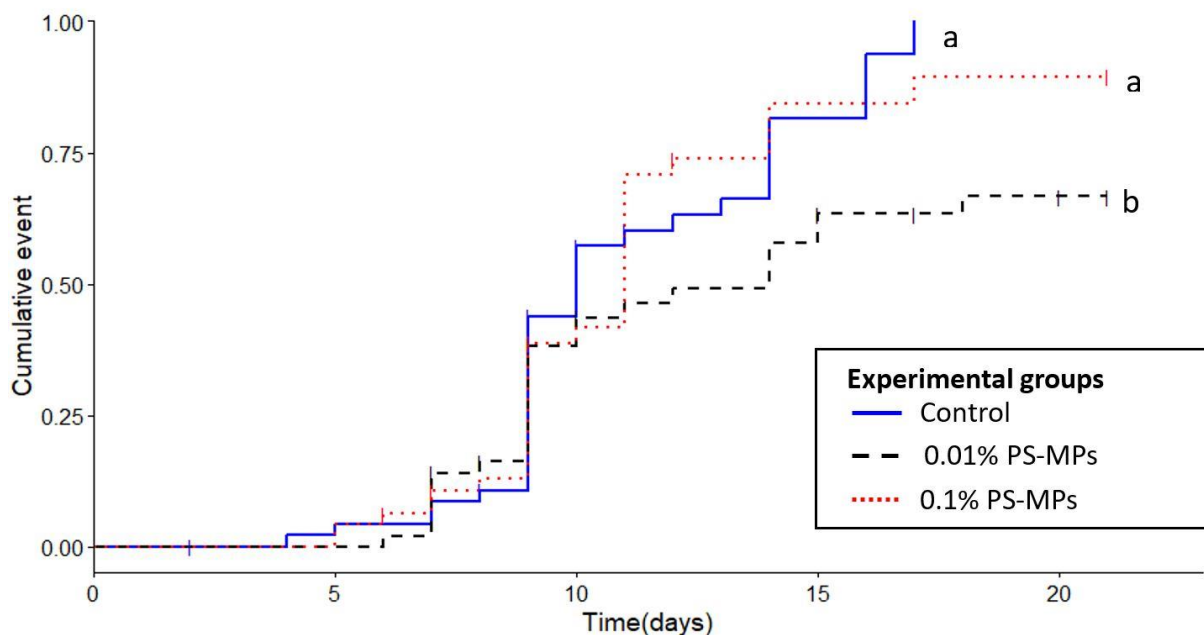


Figure 30: Kaplan-Meier curves of the *C. lefebvrei* larvae development from the first moult (day 0 in the plot) to the second moult, corresponding to the control, 0.01 % and 0.1 % exposed second stage larvae. Different letters indicate significant differences between the exposure modalities. The curves show breaks (|), when a censoring event occurs.

IV B.3.4. Phenoloxidase activity

The PO activity for each group is summarized in figure 31. Significant increases of the PO activity were observed between the controls and the 0.01 or the 0.1 % fed-larvae in the basal

PARTIE B – EVALUATION DE LA POSSIBILITE DE TRANSFERTS DE MICROPLASTIQUES DANS LES CHAINES ALIMENTAIRES–DU CONSOMMATEUR PRIMAIRE AU CONSOMMATEUR SECONDAIRE ($p = 0.032$ and $p=0.0381$ for the 0.01 % and the 0.1 % groups VS CTL, respectively) and the total ($p = 0.0021$ and $p = 0.0226$ for the 0.01 % and the 0.1 % groups VS CTL, respectively), while no difference was found between the basal and the total PO activity within any of the groups. The PO activity followed a non-monotonous concentration-response pattern, with an increased PO activity for the 0.01 % group VS CTL, and a decreased PO activity in the 0.1 % group VS the 0.01 % group.

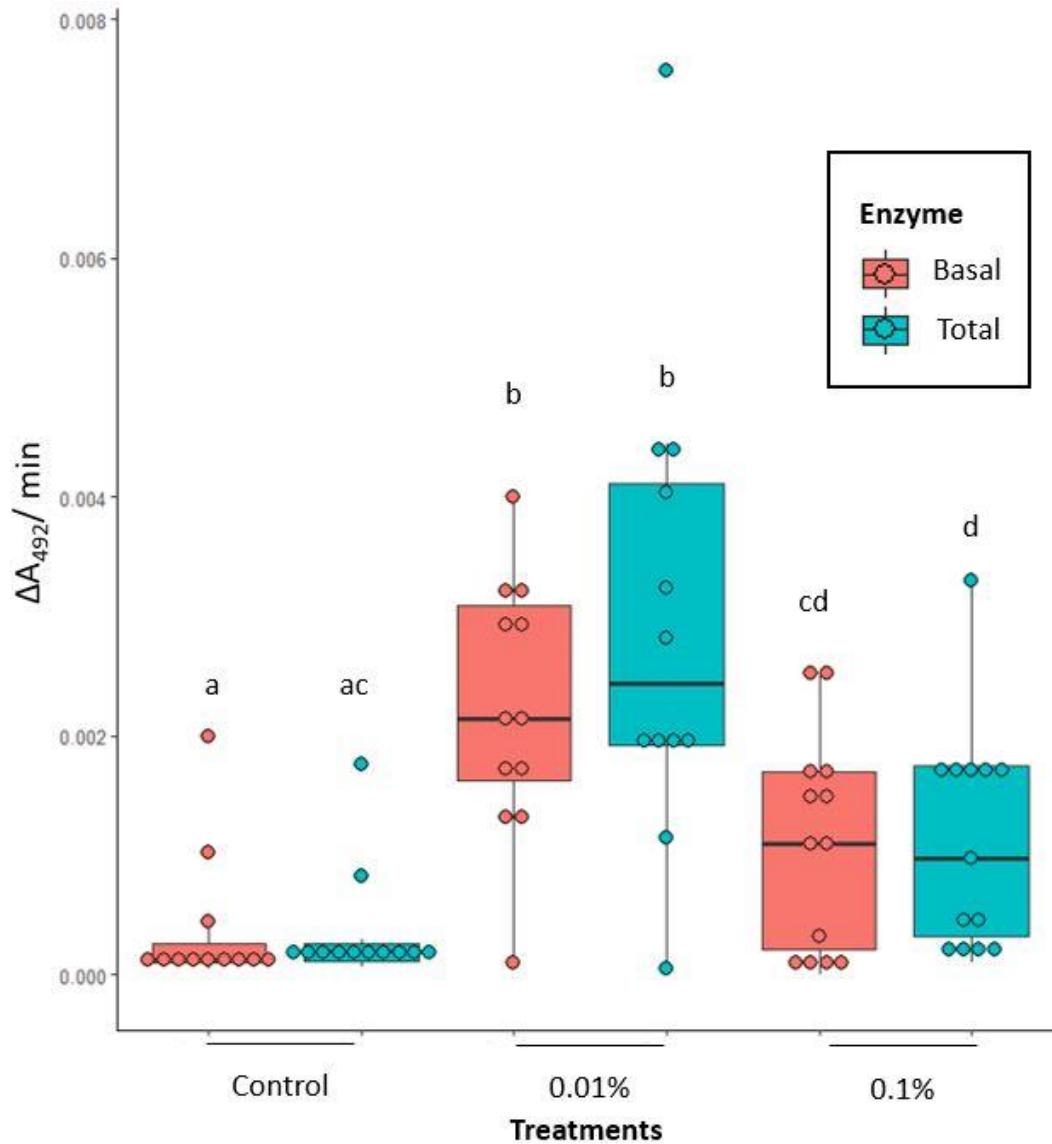


Figure 31: Basal and total plasma enzyme activities of phenoloxidase (PO) in control *C. lefebvrei* larvae ($N = 12$) and those fed with contaminated snails (*C. aspersus*) at low (0.01% PS-MPs; $N = 12$) and high (0.1% PS-MPs; $N = 13$) concentrations. Each sample represents the plasma pool of two different individuals. Enzyme activity was recorded as absorbance units per minute ($\Delta A_{492} \text{ min}^{-1}$) 7 days after the second moult. Box plots not sharing the same letter are significantly different at p -value < 0.05 .

IV B.4. Discussion

This study demonstrated that PS particles can be transferred from a food source to a primary consumer, the terrestrial snail (*C. aspersus*), and subsequently transferred from the snails to their predators (*C. lefebvreii*), the secondary consumers. This original result was obtained using an indirect detection method by rare-earth-element measurement. The advantages of such method were the avoidance of false positive detection that can occur in fluorescence imaging, as biological tissues can present autofluorescence phenomenon due to the presence of intrinsic fluorophores (Del Rosal and Benayas, 2018; Wolfbeis, 2015). Furthermore the Eu measurement in a tissue specifically indicates the presence of particles, as PS particles does not measurably swell in desorption experiment in water (Wolfbeis, 2015). Several researches have shown microplastics can be ingested by organisms when exposed by direct food exposure (Colpaert et al., 2022; Cui et al., 2022; Huerta Lwanga et al., 2017; Rodrigues et al., 2023). Microplastics have also been found both in aquatic (for instance in zooplankton, mussels, fish, waterbirds and cetaceans; Nelms et al., 2018; Smith et al., 2018; Zheng et al., 2023) and in terrestrial (for instance in earthworms, pillworms, crickets, locusts, slugs or spiders; Wang et al., 2022) wild animals, though in such cases, multiple exposure routes are to be envisaged. In terrestrial ecosystems, evidences of trophic transfer in field experiment are scarce (Huerta Lwanga et al., 2017), but MPs have been found in the digestive tract of carnivorous terrestrial birds, suggesting a MP trophic transfer (Baho et al., 2021a). Laboratory experiments focusing on terrestrial trophic transfer are now increasing, but they are still mainly focused on aquatic ecosystems (Chae et al., 2018; Da Costa Araújo et al., 2020; Elizalde-Velázquez et al., 2020; Farrell and Nelson, 2013). However, research efforts have already been made: Da Costa Araújo and Malafaia, (2021) have done an original preliminary study on the trophic transfer of $35 \pm 18.17 \mu\text{m}$ PE MPs on aquatic organisms (fishes fed on frog tadpoles directly exposed via water) versus mice directly exposed to MPs via water. Using Nile red staining and fluorescent microscopy, they retrieved PE MPs both in directly exposed tadpoles, indirectly exposed fishes and in livers of mice exposed via contaminated fishes, as well as in directly exposed mice livers. They also noticed a decrease in the PE size in the mice vs in the lower levels of the food chain, indicating that only the smallest size can transfer from mice GI tract to the liver. They also observed a linear correlation between the MP accumulation with a decreased locomotor activity and a higher anxiety rate in mice. Another study reported the

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possible MP transfer from terrestrial to aquatic ecosystems as well, with lettuce grown on MPs (PS or PVC, 250 nm) contaminated soil, black soldier fly larvae fed on the previously contaminated lettuce leaves, then given to feed fishes in a short-term experiment (Abdolahpur Monikh et al., 2022). They showed that only 3 % of the MPs were transferred from the soil to the lettuce shoot depending on the polymer, but a high MP proportion was transferred from the plant to the insects (with a MP bioaccumulation in insects) and to the fish. Their study highlighted the absence of MP biomagnification from plants to fishes during the experiment, which does not negate the possibility of MP biomagnification over a longer timeframe. This is consistent with the results of our present study, which shows between 28 % (for the 0.1 % Eu-PS group) and 42 % (for the 0.01 % Eu-PS group) MPs transferred from the food to the snail, and 0.4 to 0.5 % of the MPs transferring from the snail to the larva. Their study is also consistent with our results, with a decrease in the number of particles along the food chain on the basis of the short-term experiment encompassing the larval stages of the carabid beetles, leading to TTF under 1, showing that the transfer was not accompanied by biomagnification during the experiment. However, the TTF for the carabid beetles may be underestimated in our study. This underestimation can come from the loss of the contaminated haemolymph during their dissection to remove the GI tract, such biological compartment possibly being much more contaminated than the other tissues, as it can receive the possibly internalized particles and distribute them to the different organs. These results evidenced the possibility of MP exposure of a longer-lived predator foraging and feeding on contaminated preys. In this study, it is important to distinguish the TTF in the snail, which correspond to a “Trophic Uptake Factor”, from the TTF in the carabid beetles, which correspond to a “Truly Trophically Assimilated Factor”. This difference is fundamental, as the latter really reflect an internal contamination of the individuals, allowing our study, as far as we know, to be one the few demonstrating a real microplastic trophic transfer in internal tissues of the animals through a food web contamination. When the microplastics are small sized, they have the ability to be absorbed by plants via the plant roots, and then translocate from the roots to shoots or fruits in plants (Abdolahpur Monikh et al., 2022; Oliveri Conti et al., 2020). They then can be ingested by plant consumers, and transferred from the GI tract to other organs in animals, thus allowing MPs to directly exerts nefarious effects on the organs (Amerah et al., 2020; Farrell and Nelson, 2013; Wang et al., 2022). Thus, micro- and nanoplastics can accumulate in the biota and be transferred via predation to upper levels in

PARTIE B – EVALUATION DE LA POSSIBILITE DE TRANSFERTS DE MICROPLASTIQUES DANS LES CHAINES ALIMENTAIRES–DU CONSOMMATEUR PRIMAIRE AU CONSOMMATEUR SECONDAIRE the food chains. However, studies have generally shown that biomagnification isn't likely to occur. Nevertheless, data collected largely relies on the MP number in GI tracts on the animals, which cannot be considered to the internal compartment of the organism, reflecting a dynamic process whereby particles are passing through the organism, and not in the tissues. This represent a knowledge gap in assessing bioaccumulation and trophic transfer, as suggested by Gouin, (2020).

Another interesting result is the PS concentrations in the exuviae, with 12-16 times more PS particles in the exuviae than in the soft tissues. This phenomenon can correspond to a PS detoxification strategy from the carabid beetles. The ecdysis was already shown to be a metal detoxification mechanism in hexapods and crustaceans (Dallinger, 1993; Ramos and Leite, 2022), and the particle size can theoretically allow them to translocate. Nonetheless, PS being particulate matter, the exuviae-incorporation-mechanisms should be different than metal ions ones, and therefore needs to be further elucidated. Here, considering an already described metal detoxification mechanism using the ecdysis in hexapods, and the comparative higher MP load in the larvae exuviae, we highlighted the ecdysis as a potential MP detoxification mechanism in the studied larvae. Finally, even if such physiological processes can participate in MPs detoxification mechanisms in hexapods, the total amount of MP to take into consideration in the beetles with their exuviae just before moulting may not be higher than if regarded with solely the soft tissues amount, as the exuviae only participate marginally in the total mass balance of the animal, compared to the soft tissues. This could indicate an increase in MP internal concentrations (or at least a steady concentration) in larvae exposed to MPs during their lifespan, despite MPs detoxification strategies. This MP accumulation can then pose a risk toward their predators, and the upper levels of the food webs.

Other studies have focused on the transfer of MPs from plants to animals in terrestrial ecosystems. Chae and An (2020) assessed the effects of 20 nm PS beads on mung beans, and the effects of beads trophic transfer to the *Achatina fulica* snails. They observed PS accumulation in plant leaves, associated to decreased growth both in mung beans and in snails and GI tract disorders in snails (tissue laceration and decreased gut microbiome abundance); however, no PS measurement was done in the snail to assess the extent of the transfer. Li et al. (2023) went a step further, with an experiment of lettuce grown on 50nm PS-contaminated Hoagland solution at concentrations ranging from 0.1 to 1000 mg/L as a food supply for

PARTIE B – EVALUATION DE LA POSSIBILITE DE TRANSFERTS DE MICROPLASTIQUES DANS LES CHAINES ALIMENTAIRES–DU CONSOMMATEUR PRIMAIRE AU CONSOMMATEUR SECONDAIRE *Achatina fulica* snails. They retrieved PS particles by pyro-GC-MS in all the tested concentrations in lettuce (except the control) both in root and shoot, indicating a root to shoot translocation, and PS in digestive tract of the animals after 2 days non-contaminated feeding, and to a lower extent in the digestive gland of all exposed groups except from the 0.1 mg/L group, associated with a decreased shoot biomass at the higher concentration and differences in the snail feeding rate. However, they also observed decreased PS concentrations in the higher level of the food chain compared to the lower level, which is still consistent with literature.

The PO system in invertebrates participates in several functions, such as immunity, wound healing and tissue repairing, moulting or pigment synthesis (González-Santoyo and Córdoba-Aguilar, 2012; Lu et al., 2014). In the immunity context, the PO-system is a humoral defense which participates in the melanization process, where the PO inactive zymogen present in plasma, the prophenoloxidase (pro-PO) is activated into PO after the recognition of potentially harmful pathogens (such as bacteria, fungi, viruses or endoparasites). This activation triggers a molecular cascade pathway finally leading to both the polymerization of melanin to encapsulate the pathogens, and the production of reactive and toxic compounds to eliminate the pathogen (Cerenius et al., 2008; Hillyer, 2016). Thus, the plasmatic PO activity is a marker of the immunity status of the animals. In *C. lefebvrei*, the PO activity have been demonstrated to fluctuate according to the gender and the developmental stage of the animals (Giglio et al., 2016; Giglio and Giulianini, 2013), depending on the needs of the animals to invest in immunity to maximize their fitness. Exposure to metal(loid) contaminants have been shown to exert nefarious decreasing effects on the PO activity of *C. lefebvrei* (Talarico et al., 2014), pointing to a possible decrease of the immunity competence of the exposed animals. Here, we did not observe a decrease in the immunity competence of the animals, but an increase in the plasmatic PO activity, both basal (reflecting the already active plasmatic PO) and total (reflecting the already active PO and the pro-PO zymogen levels in the animal plasma). This result supposes the possible triggering of an immune response of the animals after recognition of the PS as a stranger body in the animal. Another explanation can be the triggering of an inflammatory reaction (from a physical or a chemical interaction of MPs with the biological tissues), which is typically the case for small MPs and NPs contaminations, as shown in other biological models (Hu et al., 2021; Jing et al., 2022; Yin et al., 2023). Such exposure could have

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then triggered inflammation both in the GI tract and in the hemocoel of the exposed individuals, also increasing the PO activity to repair the tissue damages caused by the PS particles.

Concerning the effects of the PS contamination of the carabid beetles, the results showed no specific effect of the PS particles neither in mortality nor in the time required for the larvae to develop. However, intermediate PS concentrations impacted the moulting rate of the larvae, while higher PS concentrations did not. Furthermore, the PS contamination of the larvae also led to specific changes in the PO activity of the exposed animals, with intermediate PS concentrations posing higher effects than higher concentrations. Larval development is related to a number of factors, including temperature, nutritional intake and health status. For a given nutritional balance, trade-offs are made between immunity and other life-history traits (Cotter et al., 2011) like growth, or in adults, reproduction. When animals were exposed to PS particles, their immune functions were affected and upregulated, as previously shown. Such triggering of the immune function may have led to an imbalance in the energy allocation budget of the animal, the PO pathway being energetically costly, diminishing the resources allocated for growth and thus leading to a decreased molting rate in the exposed animals.

Finally, intermediate PS concentrations posed negative effects for both the moulting rates and the PO activity of the animals, while higher PS concentrations did not have such effects. This phenomenon corresponds to a non-monotonous concentration response of the biological markers of the animals toward the PS exposure. Polystyrene particles here could have induced an activation of the immune system of the animals and/or an inflammation at the intermediate PS concentration, imposing energetic trade-offs to the animals to manage the inflammatory process (Silva et al., 2021; Wright et al., 2013). Such energetic trade-offs leading to less energy invested in growth, and thus to lower moulting rates. The decreased PO activity in the higher PS concentration compared to the intermediate one could be related to a particle clustering in the animal gut at higher concentrations, leading to a decreased transfer to the hemolymph, even if in the present case this is unlikely, owing to the increased PS tissular contamination in the higher exposure concentration. Another explanation could be the downregulation of the PO-pathway, concomitant with the upregulation of other immune responses at higher PS contaminations. Such hypothesis is more likely to occur to enhance the animal fitness, as the maintenance of the PO pathway is energetically consuming, and it can

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also release toxic compounds which can in turn negatively affect the animal (González-Santoyo and Córdoba-Aguilar, 2012). Nonetheless, such biphasic response patterns falls under the definition of hormesis (Calabrese and Baldwin, 2002), and highlight a growing body of evidences on the hormetic responses in ecotoxicological models for the characterization of the effects of MPs (Rix et al., 2022; Sun et al., 2021). However, the complex underlying biological processes are still to be explained to fully decipher the effects of the MP contaminations on the terrestrial biota.

IV B.5. Conclusion

Through an indirect method using Eu-PS MP transfer along the food chain flour-snail-carabid beetle was showed without MP biomagnification with the increasing level in the food. Tissue internalization of PS was evidenced in the carabid larvae, as the GI tract of the carabid beetles were removed. This is however not inconsistent with a potential biomagnification in natural environment, whereby multiple contamination pathways, during whole lifecycle exposures can occur. The MP transfer from preys to predators in presence of MP contamination seems to trigger non-monotonic concentration responses in the animals, with an increased PO activity and decreased moulting rates in the animals exposed to intermediate PS concentrations, suggesting an increased immunity response and/or tissular inflammation, and subsequent energy reallocation to manage the effects of the contamination.

The innovative indirect MP quantification method used in this experiment allowed us to cope with the technical analytical issues of small MP screening in large biological sample sets encountered with traditional direct estimation methods. Furthermore, this method allowed us, and from our knowledge, for the first time, to detect and reliably quantify real trophic transfer of MPs internalized in animal tissues, following a food web contamination. Finally, this allowed us to link an hormetic effect triggered by a MP contamination on the animal condition with reliable, quantified data on the internal exposure of MP of the animal internal tissues.

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Partie C : Discussion générale,
conclusions et perspectives

I. Discussion générale

La pollution des milieux par les MPs est aujourd'hui prise en compte, et des efforts de régulation visant les plastiques, aux échelles internationales et régionales ont été mises en œuvre ou sont en cours d'élaboration (régulation de la génération des déchets plastiques à tous les stades du cycle de vie du plastique par les Nations Unies, bannissement des microplastiques primaires pour certaines applications commerciales par l'Union Européenne). Cependant, les textes ne prennent que peu en compte la réduction de l'émission des microplastiques secondaires, ni l'étude de leur impacts environnementaux à des fins de régulation (Björkman, 2023; Commission Européenne, 2021; United Nations Environment Programme, 2022), la priorité étant pour le moment mise sur la réduction de l'utilisation et des rejets des macroplastiques et microplastiques primaires. Pourtant, à l'instar des contaminations par les polluants chimiques, la présence de MPs dans les sols conduit à leur dégradation en changeant leurs conditions physicochimiques, en perturbant la structure de leurs communautés et engendre des conséquences écotoxicologiques de par leurs effets sur la santé des organismes (Bodor et al., 2024; Mondol et al., 2024). Plusieurs raisons rendent difficile l'étude des MPs et l'utilisation des données obtenues à des fins de protection et de préservation de l'environnement :

- la difficulté technique, liée à des verrous méthodologiques, pour évaluer la présence des MPs dans les différents compartiments biotiques et abiotiques (récupération, séparation/purification, caractérisation/quantification),

- le nombre d'espèces modèles utilisées pour caractériser les potentiels effets toxiques des MPs sur le biote soumis à leur présence dans leur environnement, la plupart du temps restreint à un nombre limité d'espèces,

- la difficulté à déterminer les causes de la toxicité des MPs, seuls ou en association, sur les milieux et le biote (nature particulière, variations de concentrations, natures polymériques, tailles, contaminants adsorbés, additifs, éco-coronas, pathogènes, interactions avec le sol et son contenu),

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- les résultats hétérogènes concernant la qualification des effets des MPs sur le fonctionnement des milieux et sur la santé du biote, liés à la multiplicité des paramètres à prendre en compte cités précédemment,

- la difficulté d'évaluer, en étude *in situ* comme *ex situ* en laboratoire, la charge réelle en microplastiques du biote (quantité internalisée, à l'exclusion du contenu du tube digestif), liée aux verrous méthodologiques cités précédemment,

- l'absence actuelle de standardisation, à la fois des méthodes de caractérisation et quantification des MPs, et des méthodes d'évaluation des effets toxiques des MPs.

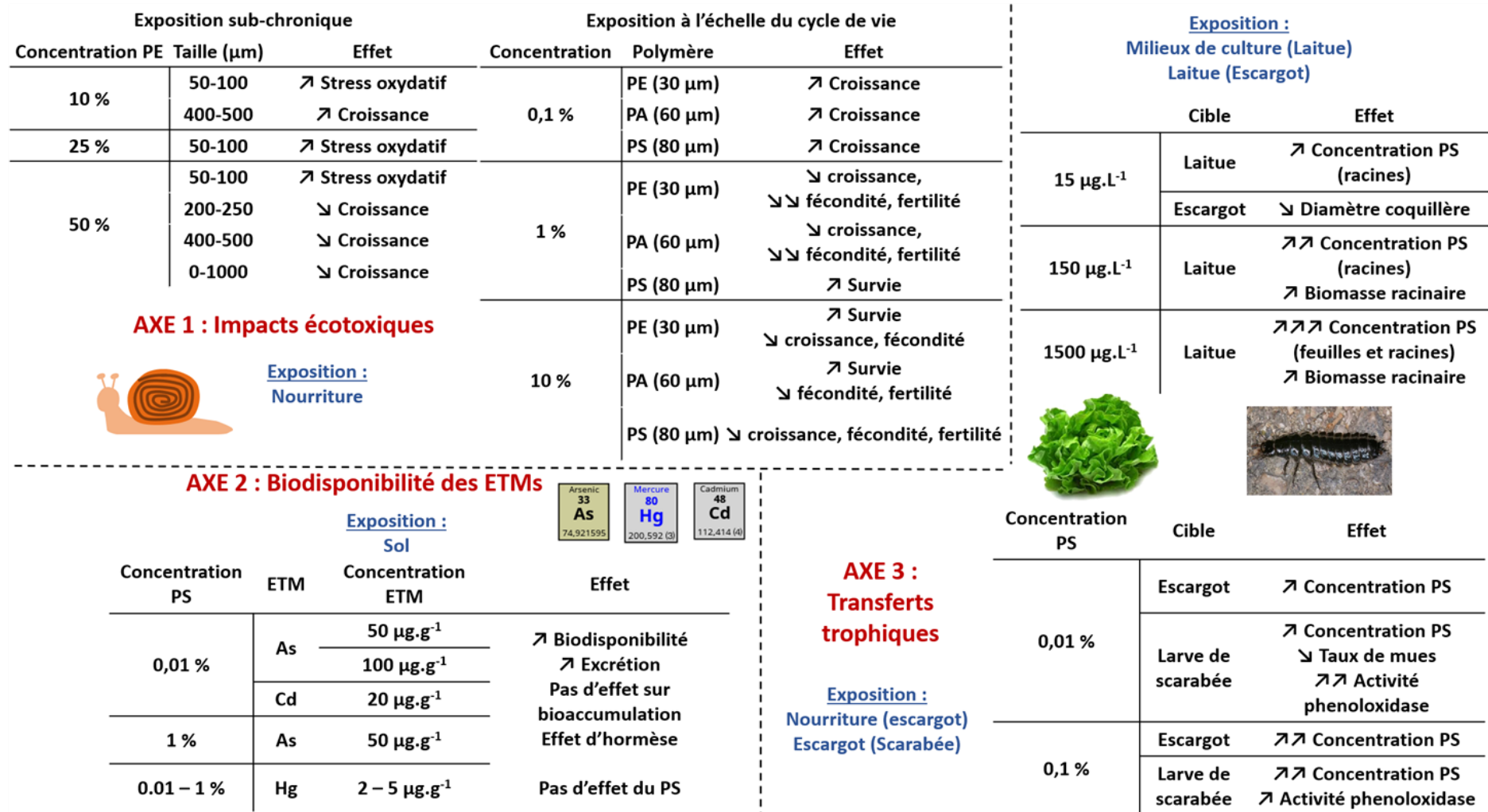
Dans le cadre des efforts de recherches à mener sur le sujet, la présente thèse visait à acquérir des connaissances pour mieux appréhender la potentielle toxicité des MPs envers la faune du sol à travers une batterie d'expérimentations *ex situ* sur un modèle biologique novateur pour l'étude des effets des MPs. Ce modèle biologique, moins inféodé au sol que d'autres espèces (*e.g.* vers de terre etc...), est complémentaire de ces espèces dans l'étude de la qualité des sols, de par sa position originale à l'interface entre le sol, les plantes et l'atmosphère.

La toxicité des MPs lors d'exposition court terme via la nourriture a été évaluée, en tant qu'étude préliminaire, afin de déterminer les effets de gradients de tailles et de concentrations d'un polymère sur des escargots juvéniles. Sur la base de ces recherches, un autre objectif était de caractériser des différences d'effets toxiques de 3 polymères sur l'escargot, sur des gammes de tailles similaires et dans des concentrations environnementales, à l'échelle du cycle de vie de l'animal. Des approches de modélisation ont été utilisées pour déterminer les différences d'effets sur des indicateurs à l'échelle individuelle (survie, croissance, reproduction). Les impacts associés aux MPs ont également été étudiés, avec la mise en place d'une expérimentation portant sur l'effet des MPs sur la disponibilité environnementale et de la biodisponibilité de différents ETMs pour l'escargot depuis le sol. Des approches cinétiques ont été envisagées dans ce travail afin de révéler des processus dynamiques d'assimilation et d'élimination des métaux qu'une approche de quantification de la seule bioaccumulation après une durée d'exposition définie n'aurait pas rendu possible. Enfin des approches innovantes de recherche des MPs dans des tissus biologiques, via l'utilisation de particules marquées, ont été utilisées pour caractériser la capacité des MPs à diffuser dans l'environnement via les chaînes alimentaires. Un récapitulatif des

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expérimentations avec leurs principaux résultats est fourni en figure 32. Dans cette discussion générale, les principales avancées scientifiques seront présentées, ainsi que les limites de ce travail et les perspectives de recherches associées.

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Exposition :
Nourriture



Arsenic 33 As 74,921595	Mercur 80 Hg 200,592 (3)	Cadmium 48 Cd 112,414 (4)
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Figure 32: Schéma récapitulatif des expérimentations effectuées durant le travail de thèse, avec les principaux résultats.

I.1. Apports scientifiques et méthodologiques de la thèse pour la compréhension des impacts potentiels des microplastiques

I.1.1. De l'utilisation combinée des études *ex situ* en exposition sub-chronique et à l'échelle du cycle de vie pour l'évaluation de l'impact des microplastiques

L'analyse des effets écotoxiques des MPs en laboratoire révèle toute une gamme de réponses sur les paramètres étudiés chez l'escargot. En effet, les paramètres individuels étudiés (croissance, mortalité) montrent des variations selon les conditions expérimentales (Figure 32). Pour un même polymère (ici le PE), la croissance est inversement proportionnelle à la concentration, à la fois sur la base de l'exposition sub-chronique (inhibition de croissance à 50 %, stimulation de croissance à 10 % pour une taille du PE de 250-500 μm) et de l'expérimentation chronique (inhibition de croissance à 10 %, stimulation de croissance à 0.1 % sur une taille de 30 μm). Les deux expérimentations comprenaient une concentration à 10 % en PE, cette concentration donnant des effets différents sur le même paramètre (la croissance) entre les deux études. Ces différences ne proviennent pas du protocole expérimental, dans les deux cas les escargots étant des individus juvéniles âgés d'un mois au début de l'exposition, et la mesure se faisant en période de croissance linéaire. Cette différence d'effet sur la croissance des individus est ici à mettre en relation avec la taille des particules, ce qui confirme les données issues d'autres modèles biologiques (Bodor et al., 2024). L'exposition sub-chronique a montré une augmentation du stress oxydatif chez l'animal uniquement pour les tailles de particules les plus petites de l'expérimentation, soulignant ici l'augmentation des effets toxiques des MPs avec la diminution de leur taille (Cui et al., 2022; Dissanayake et al., 2022). Concernant la survie des individus, les expérimentations sub-chroniques comme chroniques n'ont pas montré d'effet néfaste des MPs sur ce paramètre. Ainsi, les différentes durées d'exposition donnent sur la mortalité les mêmes trajectoires d'effet. Ceci pourrait laisser à penser qu'une exposition sub-chronique peut suffire pour déterminer les effets écotoxiques des MPs dans un futur cadre réglementaire. Cependant, comme montré en chapitre II, l'exposition de l'escargot aux MPs à l'échelle du cycle de vie révèle une amélioration de la survie de l'animal après le deuxième mois d'exposition. Ces effets sont à mettre en relation avec une diminution de la capacité des individus à se reproduire, et aux différences d'investissements énergétiques entre maintien de l'activité de

l'organisme, lutte contre les effets toxiques des MPs, et reproduction. Ainsi, l'étude des effets chroniques des MPs sur l'escargot nous a permis de montrer l'intérêt de ce type d'exposition à long terme, en mettant en évidence des effets sub-léthaux chez l'animal, portant sur les aspects de reproduction (le paramètre biologique le plus impacté par les MPs chez l'escargot dans nos travaux), la fitness des individus, et potentiellement en lien avec des perturbations endocrines.

Les expérimentations portant sur l'écotoxicité des MPs envers l'escargot nous ont ici permis de 1) générer des données sur les effets écotoxiques des MPs sur un modèle biologique jusqu'alors inusité dans ce cadre, et donc d'augmenter le nombre de modèles biologiques utilisés dans l'évaluation du risque environnemental des MPs, et de 2) déterminer les effets de plusieurs polymères plastiques sur des marqueurs individuels reflétant la santé des individus, et de proposer des pistes d'explications mécanistiques (possible perturbation endocrine, changements dans les compromis énergétiques, changement de composition du microbiote intestinal, ...) à explorer dans de futurs travaux. Finalement, le paramètre biologique le plus impacté lors de nos travaux est la reproduction des individus. Ce paramètre est d'importance, car il permet de souligner de possibles perturbations des processus biologiques sous-jacents. De plus, une réduction de la reproduction chez des individus exposés aux MPs peut fortement impacter la dynamique de population de l'espèce considérée sur la zone géographique concernée. Ces travaux montrent donc également l'intérêt de l'utilisation d'expositions à l'échelle du cycle de vie, le paramètre biologique le plus impacté par les MPs n'étant pas spécifiquement la survie, mais pouvant être la reproduction des individus, celle-ci n'étant disponible que lors d'exposition durant le cycle de vie de l'animal.

I.1.2. Effets des microplastiques sur le comportement de co-contaminants

De nombreuses études se sont penchées sur les effets des MPs sur la disponibilité environnementale des ETMs dans les sols et leur bioaccumulation dans le biote inféodé au sol, ainsi qu'aux mécanismes physiques régissant les effets des MPs sur le comportement des ETMs (Liu et al., 2024; Song et al., 2022). Ces études montrent, selon les conditions expérimentales, des augmentations, des diminutions, ou une absence d'effet spécifique des MPs sur la disponibilité environnementale et/ou la bioaccumulation des ETMs dans le biote.

Le travail effectué dans cette thèse sur les interactions MP-ETMs est donc en accord avec une partie de la littérature. En effet, dans nos conditions expérimentales, les MPs n'auraient que peu d'influence sur la disponibilité et la bioaccumulation des ETMs pour *Cantareus aspersus*, celles-ci étant surtout dépendantes de la concentration en ETMs dans le sol. Cependant, grâce aux approches toxicocinétiques utilisées dans cette thèse, nous avons pu mettre en évidence des effets des MPs tant sur la biodisponibilité environnementale des ETMs que sur leur excrétion chez l'escargot, malgré une influence limitée sur leur bioaccumulation. Dans le cas de co-contamination, cette influence des MPs sur ces paramètres apparaît importante à considérer, celles-ci modulant (en association avec les capacités de l'escargot à séquestrer les ETMs) la fraction d'ETMs dans l'individu pouvant interagir avec leurs cibles biologiques, et pouvant donc exercer des effets écotoxiques sur l'escargot (en terme de survie, croissance ou reproduction, *i.e.* leur biodisponibilité toxicologique ; Gimbert et al., 2008).

Un certain nombre d'études ont montré le rôle des MPs comme vecteurs d'ETMs (Frost et al., 2022; Tariq et al., 2024; Verla et al., 2019; Wu et al., 2023), ceux-ci agissant comme un « cheval de Troie » (adsorption / désorption d'ETMs sur ou depuis les MPs ; Hildebrandt et al., 2021) dans les milieux. Dans le cadre de notre travail, l'effet des MPs semble plutôt « indirect », ceux-ci modifiant plutôt la bioaccessibilité des ETMs dans le tractus digestif de l'animal. Ces approches apportent donc des éléments supplémentaires en faveur de l'hypothèse d'une influence des MPs sur le devenir des ETMs dans le biote, en éclairant les processus dynamiques internes qui dirigent la bioaccumulation des ETMs en présence des MPs dans le sol.

I.1.3. Transfert trophique des microplastiques

Un autre impact potentiel associé aux MPs est leur transfert dans les chaînes alimentaires. Ainsi des MPs initialement présents dans l'environnement peuvent se disséminer dans le biote et exercer de potentiels effets toxiques non seulement chez les organismes du sol, mais également chez leurs prédateurs et plus largement à toute la faune terrestre, voire l'Homme également (Nair and Perumal, 2022; Rose et al., 2023). Ce travail de thèse a montré que malgré l'absence de biomagnification des MPs depuis une source alimentaire (salade ou escargot) vers un consommateur (escargot ou larve de scarabée) dans nos conditions

expérimentales, une internalisation tissulaire de MPs était possible dans des consommateurs se nourrissant sur des sources contaminées. Les études existant sur ce sujet montrent dans leur ensemble une contamination du biote par les MPs à tous les niveaux trophiques, des producteurs primaires, aux consommateurs primaires, secondaires et apicaux (Nelms et al., 2018; Smith et al., 2018; Zheng et al., 2023). Cependant, la plupart des études analysent les contenus des tubes digestifs, ou recherchent les MPs dans les tissus en se basant sur des méthodes de détection visuelle (microscopie). Bien que ces techniques puissent être pratiques pour quantifier des MPs dans des échantillons biologiques provenant d'expérimentation *in situ* sur des MPs inconnus *a priori*, elles présentent deux principaux inconvénients. Premièrement, la quantification visuelle tend à sous-estimer la contamination, seuls les MPs les plus gros étant facilement repérables, le risque de ne pas être détecté allant de pair avec une diminution de la taille des particules. Deuxièmement, bien que l'analyse du contenu digestif soit une méthode efficace pour retrouver des MPs dans la faune, la lumière du tube digestif n'est pas considérée comme un milieu interne mais externe, cette méthode ne permet donc pas de déterminer la charge en MP des individus car ne prend pas en compte leur capacité à être réellement internalisés. Ces méthodes permettent donc de caractériser une ingestion des MPs par le biote, mais n'autorisent cependant pas une détermination précise du transfert *stricto sensu* des MPs dans les individus.

Le travail fourni dans cette thèse a permis de proposer une voie d'amélioration pour répondre à ces limitations. En effet la méthode de quantification indirecte basée sur l'utilisation de particules de MP marquées avec une terre rare (ici l'euprium) a abouti à une estimation fiable de la charge en MP dans les individus. De plus, le retrait du tube digestif nous a permis de doser ces MPs dans les tissus internes des animaux, montrant la charge en particule internalisée dans les prédateurs de l'escargot. La fiabilité de l'estimation de charge a été vérifiée durant ce travail, le lessivage de l'euprium étant minimal (de l'ordre de 0,049 $\mu\text{g.L}^{-1}$ d'euprium pour une solution à 1500 $\mu\text{g.L}^{-1}$ de PS marqué). Ainsi ce travail nous a permis de montrer quantitativement les variations de la charge en MPs à différents niveaux d'une chaîne alimentaire et de souligner leur capacité à se transférer dans les tissus d'animaux vivant dans des milieux non contaminés, uniquement par la consommation d'items alimentaires contaminés. Cependant, bien que cette technique permette de contourner les verrous méthodologiques précédemment évoqués dans cette thèse, son principal inconvénient

provient de la nécessité d'utiliser des particules préalablement marquées. En effet, cette contrainte empêche l'extension de la technique à des échantillons environnementaux de MPs, la limitant au cadre des études en laboratoire, sur des MPs préalablement traités, et donc d'origine connue. Cette approche permet donc de fournir une preuve de concept, mais manque toutefois de réalisme environnemental.

I.1.4. Les microplastiques, une question d'hormèse ?

Comme montré au cours des chapitre précédents, et illustré en figure 32, les effets des MPs présentent des réponses non concentration-dépendantes (augmentation à faible concentration puis diminution aux fortes de la croissance, diminution plus importante de la fécondité à concentration moyenne en MP, effet plus important sur la biodisponibilité des ETMs à faible concentration en MPs, effets écotoxiques plus importants des MPs sur les prédateurs des escargots à faible concentration en MP). Ce type d'effet non monotone correspond à la définition de l'hormèse, comme étant une réponse adaptative caractérisée par des doses réponse biphasiques, directement induites ou étant le résultat de processus biologiques compensatoires suite à la perturbation initiale de l'homéostasie (Calabrese and Baldwin, 2002). Il est à noter qu'ici, le fait que l'effet soit bénéfique ou délétère ne rentre pas dans la définition de l'hormèse.

Ce type de résultat est de plus en plus rapporté dans les études portant sur les effets écotoxicologiques des MPs (Rix et al., 2022; Shen et al., 2023; Sun et al., 2021), bien que la mécanistique impliquée soit encore floue. Ainsi, la contamination par les MPs a des impacts hormétiques, que ce soit sur la bioaccumulation d'autres contaminants, ou bien sur les effets biologiques chez l'escargot ou un de ses prédateurs. Ces résultats sous entendent donc, comme expliqué précédemment, une possible perturbation homéostatique chez les individus suite à leur exposition aux MPs. Cependant, les mécanismes sous-jacents (perturbation du microbiote, perturbation endocrine, induction de processus inflammatoires etc...) n'ont pas pu être explorés dans ce travail de thèse et leur étude future serait bénéfique pour mieux comprendre les modes d'actions des MPs chez l'escargot, et par extension dans les cibles biologiques des MPs.

Enfin, de nombreuses études portant sur l’écotoxicité des MPs envers le biote ont pour cela utilisé des concentrations élevées, et dans certains cas extrêmes en MPs, au regard des concentrations que l’on peut retrouver dans l’environnement. Aussi, les futures études sur l’impact des MPs devraient donc se concentrer sur de plus faibles concentrations, environnementalement réalistes, afin d’acquérir des connaissances plus complètes et plus représentatives des effets possibles des MPs dans l’environnement. Ces études à concentrations environnementales pourraient donc ainsi également intégrer le modèle de dose-réponse hormétique dans l’évaluation des risques environnementaux liés aux MPs.

1.2. De la difficulté d’une connaissance maîtrisée des effets des microplastiques, questions en suspens et perspectives

Au début de cette thèse, certains manques ont pu être identifiés, comme le faible nombre de modèles animaux utilisés dans l’étude des effets des MPs sur les invertébrés du sols, le faible nombre de marqueurs utilisés dans les études portant sur l’écotoxicité des MPs sur les mollusques terrestres, le manque de données portant sur la toxicocinétique de polluants en co-occurrence avec des MPs chez l’animal, ou des verrous méthodologiques limitant la quantification des transferts trophiques des MPs dans les chaînes alimentaires.

Les études menées *ex situ* sur *Cantareus aspersus* dans ce travail ont apporté des informations sur l’écotoxicologie des MPs sur un modèle biologique novateur pour leur étude, d’étendre nos connaissances sur les effets des MPs sur les mollusques terrestres, nous ont permis d’élargir nos connaissances sur les mécanismes régissant la cinétique d’accumulation de métaux chez l’animal en présence de MPs, et nous a permis d’effectuer de façon fiable une mesure des concentrations internes en MPs dans des animaux à différents niveaux trophiques. Une des originalités de ce travail de thèse a été d’évaluer les effets des MPs, à de faibles concentrations, sur le long terme (*i.e.* à l’échelle du cycle de vie de l’animal). Cette approche, peu utilisée chez les invertébrés ayant des cycles de vie long (de plusieurs mois) tels que l’escargot, nous ont permis de montrer l’intérêt de ce type d’étude chez les invertébrés.

Cependant, un certain nombre de questionnements subsiste après ce travail de thèse. Les microplastiques représentent des particules couvrant une large gamme de tailles allant de 5

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mm à 0.1 μm (European Commission, 2011; European Commission. Joint Research Centre. Institute for Health and Consumer Protection et al., 2010; International Standardization Organization, 2015). De ce continuum de tailles dépend un continuum d’effets, allant d’effets majoritairement physiques (changement des conditions physicochimiques du sol, pseudo-satiété ou blocage du tractus intestinal) pour les hautes tailles vers des effets majoritairement chimiques (perturbations métaboliques, cytotoxicité) pour les petites tailles de MPs, comme montré en figure 33 (de Souza Machado et al., 2018; Kim et al., 2020; Pelegrini et al., 2023; Qi et al., 2020; Zhang et al., 2022). Ces différences d’effets dues à la taille proviennent à la fois de la plus grande capacité des grandes particules à abraser les tissus (aussi dépendant de la forme des particules), de l’augmentation de leur surface spécifique avec la diminution de leur taille, et de leur capacité à traverser les barrières biologiques pour exercer des effets délétères dans les tissus.

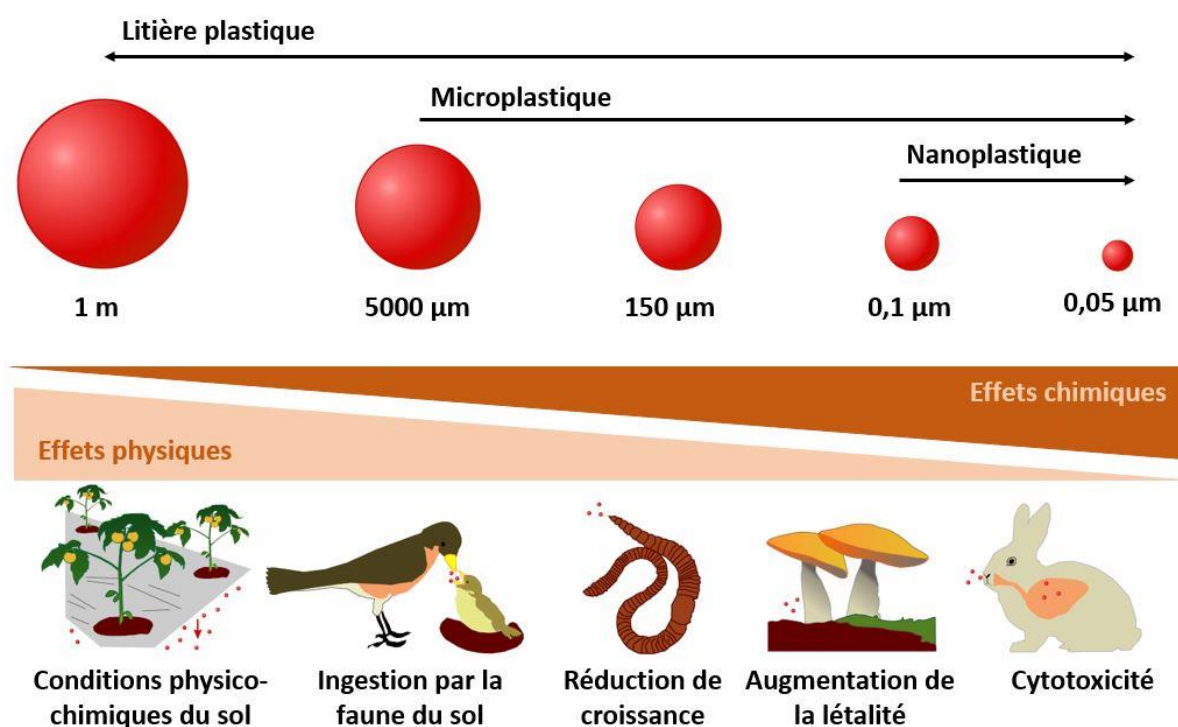


Figure 33: Variation du continuum d’effets des MPs en fonction de leur taille (adapté de de Souza Machado et al., 2018).

De ce continuum de tailles des particules de MPs découle plusieurs grandes questions qui restent à élucider. L’une de ces questions porte sur la taille correspondant à un seuil d’assimilation tissulaire après contamination du biote, particulièrement suite à l’ingestion des

MPs, cette voie étant la voie majoritaire de leur contamination (Kalčíková, 2023). En effet, il est généralement admis que les particules de plus petites tailles peuvent plus facilement passer les barrières biologiques et être internalisées. Différentes études se sont penchées sur l'internalisation tissulaires de MPs et de NPs dans des études *in vitro* (Liu et al., 2022; Stock et al., 2021), mais peu se sont penchées sur l'internalisation tissulaire de MPs dans les tissus de la faune terrestre en conditions *in vivo*. A notre connaissance, la limite de taille supérieure ayant montré une internalisation tissulaire *in vivo* est aux alentours de 20 μm (Deng et al., 2017) chez la souris. Bien que ce seuil d'assimilation puisse être le même pour la faune du sol, le manque de données ne permet actuellement pas de déterminer des seuils de tailles maximales pour l'assimilation des MPs dans ces modèles biologiques ni de déterminer la variabilité de ce seuil en fonction des espèces ou genres considérés. Ces informations sont pourtant d'utilité, les particules une fois directement internalisées au niveau tissulaire voire cellulaire, peuvent exercer des effets directement à ces niveaux, augmentant leur toxicité potentielle (Deng et al., 2020).

Ce travail de thèse s'est donc concentré sur l'étude du devenir et des impacts des MPs en prenant en compte toute une partie du continuum de tailles de ces particules (de 1 mm à 0,1 μm). Cependant ce travail de thèse s'est fait en excluant l'étude des nanoplastiques (NPs). Ce choix s'est fait sur la base des différences de comportement, fondamentales, entre les microplastiques et les nanoplastiques (pour les NPs : prise en compte du mouvement Brownien dans les matrices liquides, impossibilité d'utiliser les méthodes de détection décrites pour les microplastiques, différences dans l'internalisation, l'accumulation et les effets des NPs). Ces différences empêchent donc d'extrapoler le comportement des NPs depuis l'étude des MPs (Gigault et al., 2021). Une fois dans l'environnement, la fragmentation des MPs finit par produire des NPs, seulement l'étude de ces NPs présente de nombreux challenges, à la fois en terme de détection que de caractérisation des effets et des mécaniques impliquées (Dang et al., 2022). L'étude de ces particules représente donc un volet de recherche à part entière qui se doit donc de faire l'objet de travaux spécifiques.

Les MPs peuvent exercer des effets écotoxiques sur le biote terrestre, même en étant peu ou pas internalisés (*cf.* Partie II. Etudes expérimentales ; et figure 32), par l'intermédiaire d'effets soit physiques soit chimiques. Concernant les effets dits chimiques des MPs sur le biote, plusieurs questions d'intérêt restent encore à éclaircir.

La première question concerne la contribution des additifs dans l'effet des MPs sur le biote. En effet, aux polymères plastiques sont adjoints nombres d'additifs durant leur procédé de fabrication. Ces additifs peuvent être de différentes sortes (retardateurs de flamme, plastifiants, antioxydants, stabilisants, ...), pour baisser les coûts de production et améliorer les caractéristiques des produits finis (Li et al., 2024). Les additifs représentent environ une dizaine de milliers de molécules différentes, dont plusieurs milliers peuvent présenter un risque pour la santé humaine et environnementale, étant de possibles carcinogènes et perturbateurs endocriniens (Campanale et al., 2020; Kannan and Vimalkumar, 2021). De plus, ces additifs possèdent souvent (pour les additifs dits organiques) une grande hydrophobicité, ce qui engendre une facilité d'absorption par les tissus biologiques ainsi que de potentielles longues demi-vies et fortes bioaccumulations dans le biote (Kwon et al., 2017).

En dehors des additifs, les MPs peuvent interagir dans l'environnement avec d'autres contaminants environnementaux, qu'ils soient métalliques (*cf.* chapitre III de la section expérimentale) ou organiques. Les MPs peuvent également interagir avec les particules colloïdes du sol, ainsi que les composés organiques et inorganiques et le microbiome du sol. Ces interactions vont former, selon la taille des MPs, des microenvironnements spécifiques qui seront des plastisphères et/ou des éco-coronas, comme décrit en chapitre IV de l'introduction de thèse. Cependant, à notre connaissance, bien qu'un nombre croissant d'études se penche sur ces différents sujets, peu d'entre elles concernent l'effet des co-contaminants et additifs sur les organismes composants la plastisphère, ni sur l'impact des éco-coronas sur la disponibilité environnementale des co-contaminants. Les études de ces interactions entre MPs et des co-contaminants portent principalement sur des paramètres biologiques tels que la sélection d'agents potentiellement pathogènes, et sur la possible contamination des milieux par ces agents (Gkoutselis et al., 2021; Komorowska-Kaufman and Marciniak, 2024). Une fois dans l'environnement, les MPs peuvent entraîner des effets sur le microbiome du sol ainsi que sur leur profil métabolique (Wu et al., 2022; Zhang et al., 2023), probablement en changeant les microenvironnements disponibles et en modifiant la disponibilité en nutriments dans les sols. Une fois ingérés, les MPs peuvent entraîner une dysbiose du microbiote intestinal chez les animaux contaminés, ce qui peut potentiellement impacter l'immunité, le métabolisme et l'état de santé général de l'animal, bien que les mécanismes sous-jacents restent à élucider (Demarquoy, 2024). La question se pose donc

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concernant une potentielle dysbiose du microbiote intestinal chez *Cantareus aspersus*, qui pourrait au moins partiellement expliquer les effets chroniques des MPs chez ce modèle, en relation avec des perturbations métaboliques et énergétiques chez l’animal.

Sur la base de ces questionnements, différentes perspectives d’études peuvent être proposées à la suite de ce travail de thèse, dont :

- Déterminer la potentielle internalisation tissulaire ou cellulaire des MPs, sur une gamme de taille couvrant les petites tailles des MPs (de 0,1 à 100 μm), suite à une exposition via la nourriture et/ou le sol par *Cantareus aspersus*, pour déterminer un seuil de tailles internalisables. Les particules pouvant potentiellement être internalisées par voie digestive, cutanée et respiratoire, il serait également nécessaire de déterminer si ce seuil est dépendant de la voie d’exposition. Enfin, il serait également d’intérêt de savoir si ce seuil est généralisable à d’autres espèces ou taxons en répliquant les études sur d’autres espèces.

- l’étude des effets des MPs, seuls et en association avec d’autres contaminants (ETMs, organiques), sur différents paramètres biologiques (microbiote intestinal, stress oxydatif et inflammation, dommages à l’ADN et aux télomères, ...), pour déterminer des mécanismes expliquant la toxicité chronique des MPs sur l’escargot *Cantareus aspersus*. Pour compléter ces travaux, une étude des effets des MPs sur l’activité métabolique et énergétique chez l’escargot est à envisager, pour valider les hypothèses développées au cours de ce travail de thèse, ainsi que pour déchiffrer les mécanismes sous-jacents expliquant les perturbations de l’homéostasie de l’animal, montré par les effets d’hormèse observés. Enfin, plus largement que l’étude de l’activité métabolique, l’utilisation d’approches multiomiques (transcriptomique, protéomique, métabolomique, microbiomique, etc..) dans de futures études permettrait d’établir un profil plus complet des paramètres biologiques touchés et des mécanismes sous-jacents, ces approches ayant déjà été utilisées avec succès chez d’autres espèces (Bhagat et al., 2022).

- l’analyse du contenu en additifs dans les MPs pouvant être relargués dans différentes matrices (aqueuse, fluides gastro-intestinaux, ...), associée à des études portant sur les potentiels effets de ces additifs sur l’escargot à l’échelle du cycle de vie. Ce type d’étude permettrait de caractériser la part des additifs dans l’effet des MPs sur l’escargot.

- Enfin, le travail effectué dans cette thèse pourrait être élargi à l'étude du devenir et des impacts des nanoplastiques, à l'instar des MPs étudiés dans cette thèse. Des études complémentaires sur des natures polymériques différentes de MPs et de NPs, moins étudiées à l'heure actuelle (comme les polyvinyliques, les polycarbonates), seraient également bénéfiques afin de déterminer les impacts d'un plus grand nombre de polymères étant actuellement disséminés dans l'environnement. L'élargissement des études aux plastiques « biodégradables », comme l'acide polylactique (PLA) ou le poly(butylène adipate-co-terephthalate) (PBAT), dont l'innocuité n'est pas prouvée (Qin et al., 2021; Zimmermann et al., 2020).

II. Conclusion

L'utilisation de produits à base de plastiques a conduit à la production et à l'utilisation depuis un demi-siècle de quantités considérables de plastiques, dont une grande partie se retrouve aujourd'hui dans tous les compartiments environnementaux sous la forme de déchets, de tailles macro- à nanoscopiques, qui contaminent les écosystèmes, aussi bien aquatiques que terrestres.

Ces déchets sont destinés à se fragmenter, contaminer les environnements, relarguer des produits chimiques, et entraîner des perturbations dans les matrices environnementales et dans le biote sur un temps long. L'utilisation massive des produits à base de plastique nous a donc amenés à contracter une « dette de toxicité plastique » (Rillig et al., 2021). Cette dette de toxicité plastique ne peut être amenée qu'à augmenter pour deux raisons, qui sont i) l'augmentation des volumes de production de plastique, et donc de déchets, conduisant à une contamination croissante des milieux (à titre d'exemple, on a produit, en masse, deux fois plus de plastique que la biomasse de tous les animaux terrestres et marins réunis ; Elhacham et al., 2020), et ii) la fragmentation progressive des déchets plastiques en MPs et NPs. En effet, comme indiqué en Figure 34, le relargage de produits chimiques ajoutés de façon intentionnelle ou non intentionnelle dans les matrices polymériques augmente avec une diminution de la taille des particules. De plus, une diminution de la taille des MPs et des NPs mène 1) à une plus grande facilité pour les particules à pénétrer dans les tissus biologiques et 2) à une augmentation de leur surface spécifique, qui augmente *in fine* leurs capacités à

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interagir avec les molécules et biomolécules. Ceci conduit donc à l'augmentation de la capacité des particules à perturber le fonctionnement des milieux et à entraîner une toxicité pour le biote qui va de pair avec la diminution de leur taille et donc une augmentation de cette dette de toxicité plastique.

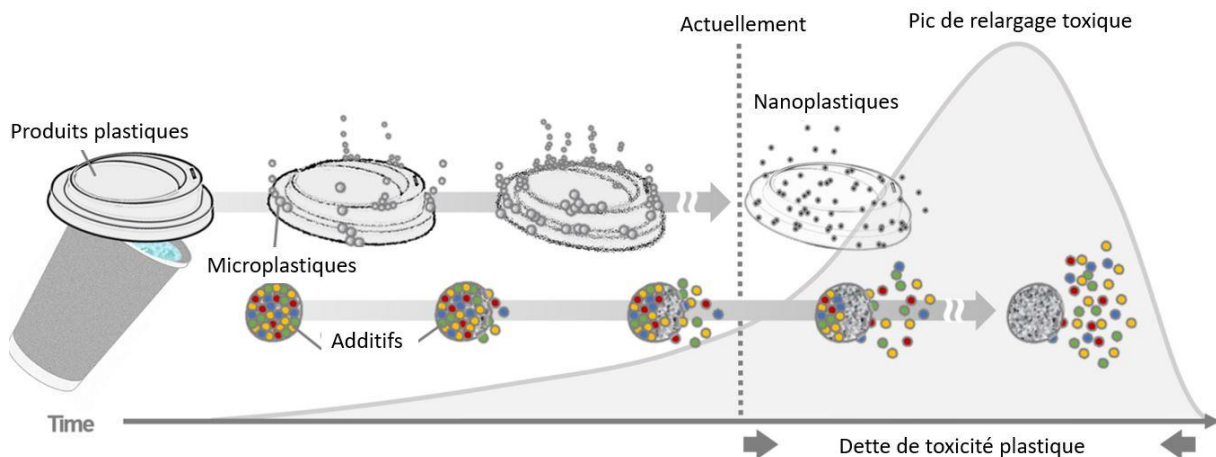


Figure 34: Illustration de la dette de toxicité plastique (d'après Rillig et al., 2021)

La contamination des milieux par les MPs va donc entraîner une perturbation du fonctionnement des milieux et une dégradation de leur capacité à assurer leurs services écosystémiques (Oliveira et al., 2019; Sridharan et al., 2021). Par exemple, ces dégradations peuvent avoir lieu en perturbant les cycles des nutriments et leur disponibilité dans les milieux (en impactant les guildes des détritivores et des décomposeurs), en diminuant la qualité physico-chimique des milieux, et notamment des milieux terrestres (effets toxiques sur les espèces ingénieuses des sols et sur les espèces pollinisatrices, impact direct des particules sur les caractéristiques physico-chimiques des sols) et peuvent s'ajouter à celles déjà causées par d'autres facteurs de stress environnementaux (réchauffement climatique, pollutions par les ETMs ou les pesticides par exemple). Ceci peut mener à une diminution des biomasses végétales et animales et notamment une diminution future de la biomasse de nos cultures vivrières. La contamination des milieux par les MPs peut aussi directement mener à la sélection de microorganismes pathogènes et résistants et donc mener à terme, à la propagation de maladie pour l'être humain. De plus, les MPs peuvent potentiellement impacter l'être humain et l'environnement de façon inattendue. En effet, les MPs participent également, par exemple, au réchauffement climatique (Parvez et al., 2024; Sharma et al., 2023). Leur production, leur dégradation et leur élimination produisent des gaz à effet de serre, leur présence pouvant inhiber la pénétration de la lumière dans les colonnes d'eau et

diminuer la biomasse algale, ou ils peuvent inhiber la croissance végétale ou encore contribuer à la formation de nuages). Le réchauffement climatique (dont l'utilisation de plastique est une des multiples causes) entraîne lui-même une amplification de la pollution plastique (dégradation précoce des produits plastiques, besoins accrus pour contrer les effets des plus hautes températures, etc... ; Wei et al., 2024).

Ainsi, dans une perspective basée sur le concept One-Health, mettant en relation la santé des écosystèmes, du biote et de l'être humain (Destoumieux-Garzón et al., 2018; Prata et al., 2021), la pollution par les plastiques et particulièrement par les microplastiques a aujourd'hui un impact significatif à la fois sur la santé de la population humaine, la santé des écosystèmes et fait peser une menace sur les services écosystémiques rendus par les différents environnements et dont nous dépendons.

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Partie E : Annexes

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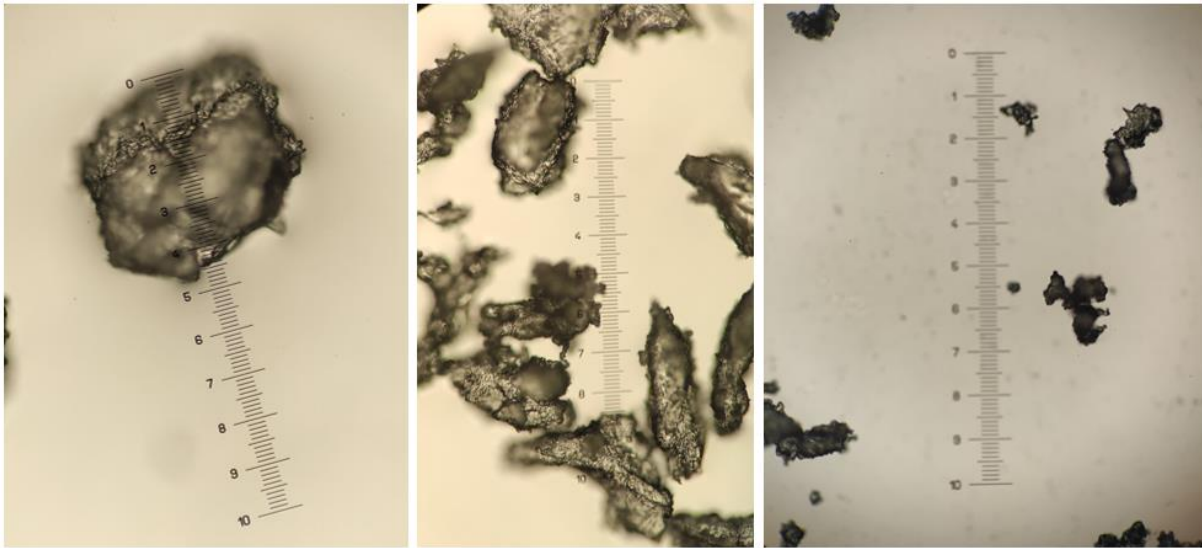
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Annexe 1 (Fig. S1) : Photographs of optical microscopic observations of the LDPE particles in the Big (left panel), Medium (middle panel) and Small (right panel) size classes. The ruler length on the photograph correspond to 1000 μm .



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Annexe 2 (Table S1) : Composition of the vegetal flour Helinove® for juvenile snails.

Net energy (kcal/kg)	Fat (%)	Cellulose (%)	Proteins (%)	Minerals (%)	Calcium (%)	Phosphorus (%)	Vitamins (UI/kg)		
							A	D	E
1429	2.6	2.5	16.5	40	13.5	1.1	25000	5000	84

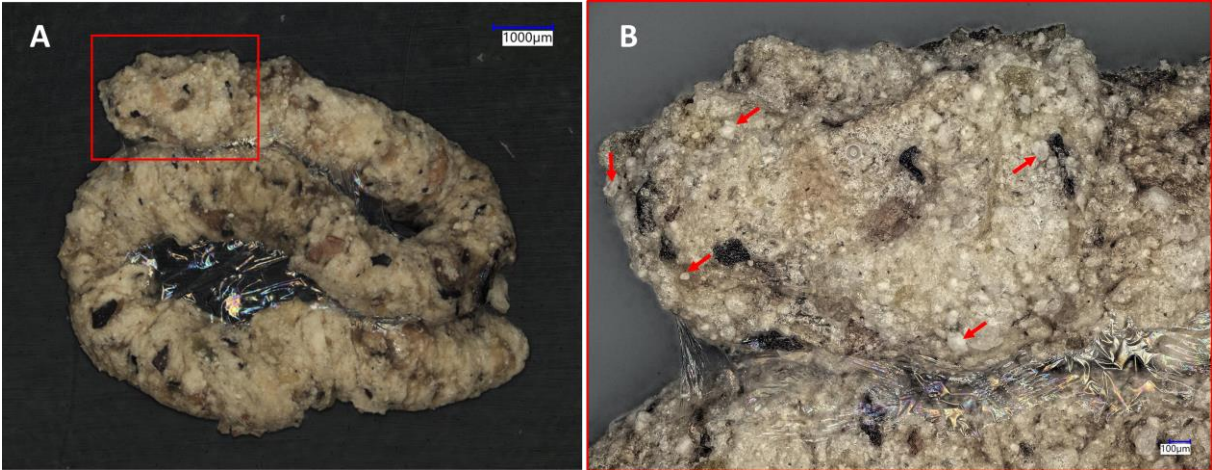
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Annexe 3 (Table S2) : Mortality percentages (%) of snails after 28 days of exposure to the different LDPE concentrations and sizes.

Exposure concentration (%)	Control	Big	Medium	Small	Mix
10	8.3	0.0	4.2	8.3	4.2
25	0.0	4.2	8.3	4.2	4.2
50	12.5	0.0	8.3	4.2	0.0

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Annexe 4 (Fig. S2) : Photographs of a feces from a snail exposed to 25% LDPE in food (Small size). A: overview (20x magnification). B: zoom (100x magnification). Some LDPE particles are marked with a red arrow.



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Annexe 5 (Table A.1.) : Modeled growth parameters Wmax (the maximal mass reached by the snails, in g), k_g (the growth constant, in d^{-1}) and I (the time at the inflexion of the growth curve, in d) for control and exposed modalities at each concentration, with the associated percentage of variation for each MP modality compared to the control in each concentration. The lowercase letters represent the significant differences for each parameter between modalities for the same concentration. The uppercase letters represent the significant differences for each parameter between concentrations for the same modality.

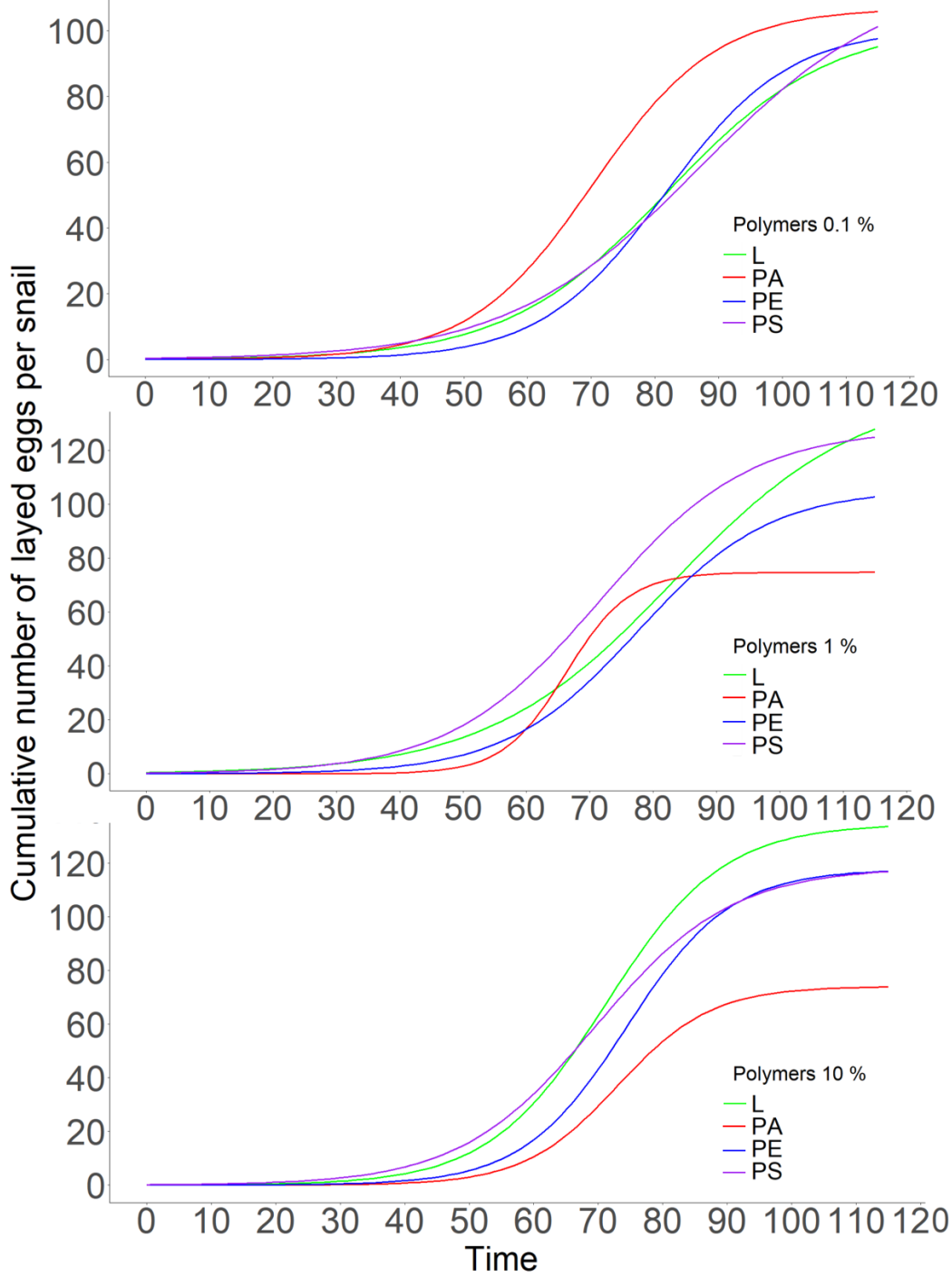
MP concentration Polymer	0.10%			
	L	PE	PA	PS
Wmax	5.17 ± 0.13	5.81 ± 0.12	5.84 ± 0.12	5.60 ± 0.12
% variation	- a / A	12.45 b / A	13.09 b / A	8.29 b / A
k_g	0.07 ± 0.006	0.07 ± 0.006	0.07 ± 0.006	0.07 ± 0.006
% variation	- a / A	15.75 a / A	9.77 a / A	10.22 a / A
I	6.61 ± 1.15	9.25 ± 0.88	8.45 ± 0.92	7.17 ± 0.98
% variation	- a / A	40.04 a / A	27.88 a / A	8.54 a / A
MP concentration Polymer	1%			
	L	PE	PA	PS
Wmax	5.73 ± 0.13	5.32 ± 0.12	5.10 ± 0.12	5.99 ± 0.12
% variation	- a / B	-7.14 b / B	-11.09 b / B	4.49 a / B
k_g	0.07 ± 0.09	0.07 ± 0.09	0.07 ± 0.10	0.07 ± 0.08
% variation	- a / A	3.45 a / A	-1.19 a / A	2.52 a / A
I	7.86 ± 1.17	6.54 ± 1.26	6.09 ± 1.38	8.87 ± 1.08
% variation	- a / A	-16.76 a / B	-23.44 a / A	12.78 a / A
MP concentration Polymer	10%			
	L	PE	PA	PS
Wmax	6.04 ± 0.17	5.36 ± 0.13	5.99 ± 0.15	5.75 ± 0.15
% variation	- a / B	-11.23 c / B	-0.91 ab / A	-4.86 b / AB
k_g	0.06 ± 0.006	0.06 ± 0.007	0.07 ± 0.007	0.07 ± 0.007
% variation	- a / A	22.67 ab / A	32.26 b / A	30.73 b / A
I	9.47 ± 1.02	7.49 ± 1.01	8.44 ± 0.83	7.60 ± 0.88
% variation	- a / A	-20.90 a / AB	-10.96 a / A	-19.75 a / A

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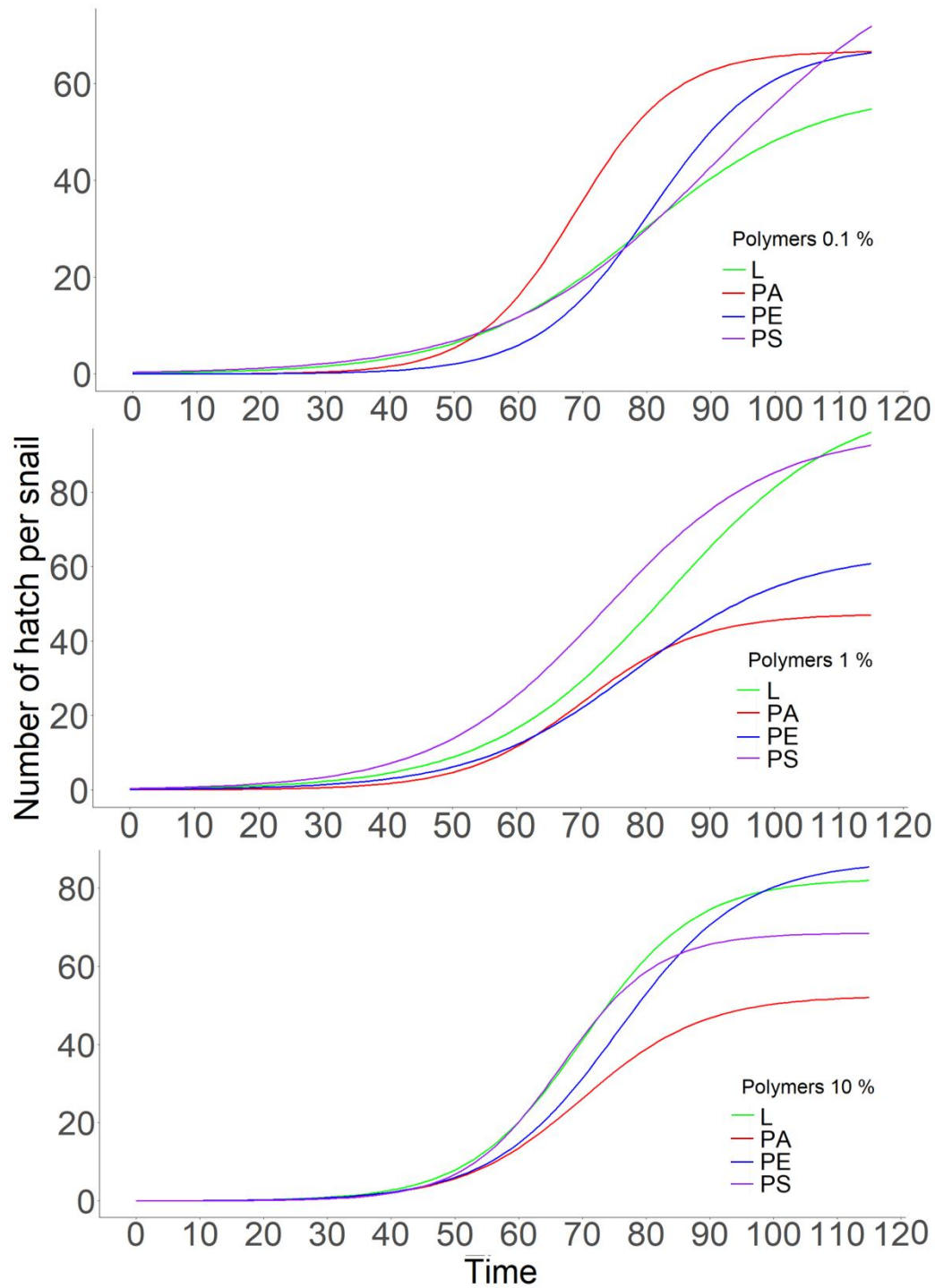
Annexe 6 (Table A.2.) : Modeled sexual maturity parameters A (% of sexually mature animal), k (the maturity acquisition constant, in d⁻¹) and I (the time at the inflexion of the curve, in d) for control and exposed modalities at each concentration, with the associated percentage of variation for each MP modality compared to the control in each concentration. The lowercase letters represent the significant differences for each parameter between modalities for the same concentration. The uppercase letters represent the significant differences for each parameter between concentrations for the same modality.

MP concentration Polymer	0.10%			
	L	PE	PA	PS
A	98.95 ± 3.82	99.45 ± 2.55	93.48 ± 2.37	97.26 ± 2.29
% variation	- a / A	0.51 a / A	-5.53 a / AB	-1.71 a / A
k	0.09 ± 0.03	0.13 ± 0.2	0.17 ± 0.02	0.17 ± 0.02
% variation	- a / A	44.44 ab / A	88.89 b / A	88.89 b / A
I	46.25 ± 1.63	37.16 ± 1.13	39.96 ± 0.98	37.49 ± 0.93
% variation	- a / A	-19.65 b / A	-13.60 b / A	-18.94 b / A
MP concentration Polymer	1%			
	L	PE	PA	PS
A	98.46 ± 2.29	91.07 ± 4.22	87.31 ± 4.88	90.24 ± 4.29
% variation	- a / A	-0.36 a / B	-4.47 a / A	-1.27 a / B
k	0.23 ± 0.11	0.19 ± 0.03	0.12 ± 0.02	0.17 ± 0.03
% variation	- a / B	11.76 a / A	-29.41 a / A	0.00 a / A
I	46.05 ± 3.09	38.09 ± 1.03	45.88 ± 1.58	39.02 ± 1.13
% variation	- a / AB	-9.80 b / A	8.64 a / B	-7.60 b / A
MP concentration Polymer	10%			
	L	PE	PA	PS
A	95.58 ± 2.39	91.43 ± 2.35	98.90 ± 2.28	92.40 ± 2.28
% variation	- ab/A	-4.34 a / B	3.47 b / B	-3.33 a / AB
k	0.15 ± 0.02	0.16 ± 0.02	0.16 ± 0.02	0.15 ± 0.02
% variation	- a / AB	6.67 a / A	6.67 a / A	0.00 a / A
I	40.82 ± 1.00	41.64 ± 0.99	40.53 ± 0.89	38.67 ± 1.01
% variation	- ab / B	2.01 a / B	-0.71 ab / A	-5.27 b / A

Annexe 7 (Figure A.1.) : Modeled number of eggs laid per sexually mature snail (representing fecundity) at 0.1 % (upper panel), 1 % (middle panel) and 10 % (lower panel) MP or lettuce control modalities, during the experiment (time in days).



Annexe 8 (Figure A.2.) : Modeled number of hatched eggs per sexually mature snail at 0.1 % (upper panel), 1 % (middle panel) and 10 % (lower panel) MP or lettuce control modalities, during the experiment (time in days).



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Annexe 9 (Table A.3.) : Mortality percentages for the control and exposed modalities at each exposure concentration. The lowercase letters represent the significant differences for each parameter between modalities for the same concentration.

Time (d)	Polymer	[MP]	Mortality	[MP]	Mortality	[MP]	Mortality
28	L	0.10%	0 ± 0 a	1.00%	2.78 ± 1.96 a	10%	8.33 ± 3.11 a
	PE		0 ± 0 a		0 ± 0 a		2.78 ± 3.11 a
	PA		0 ± 0 a		2.78 ± 1.96 a		0 ± 0 a
	PS		0 ± 0 a		0 ± 0 a		5.56 ± 3.11 a
56	L		2.78 ± 3.40 a		11.11 ± 5.01 a		16.70 ± 3.93 a
	PE		0 ± 0 a		5.56 ± 5.01 a		2.78 ± 3.93 b
	PA		5.56 ± 3.40 a		2.78 ± 5.01 a		2.78 ± 3.93 b
	PS		2.78 ± 3.40 a		2.78 ± 5.01 a		8.33 ± 3.93 ab
84	L		11.11 ± 4.17 a		11.11 ± 5.20 a		19.44 ± 4.39 a
	PE		8.33 ± 4.17 a		8.33 ± 5.20 a		2.78 ± 4.39 b
	PA		5.56 ± 4.17 a		8.33 ± 5.20 a		5.56 ± 4.39 b
	PS		5.56 ± 4.17 a		2.78 ± 5.20 a		13.88 ± 4.39 ab
112	L	13.89 ± 5.01 a	22.22 ± 5.38 a	25.00 ± 4.39 a			
	PE	13.89 ± 5.01 a	11.11 ± 5.38 ab	5.56 ± 4.39 b			
	PA	11.11 ± 5.01 a	16.67 ± 5.38 ab	8.33 ± 4.39 b			
	PS	13.89 ± 5.01 a	2.78 ± 5.38 b	16.67 ± 4.39 ab			

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Annexe 10 (Table A.4.) : Output table of the survival as a response of the egg number after generalized linear mixed effect modeling. The red lines correspond to the significant endpoints exhibiting differences (egg number, $p=2.2 \cdot 10^{-6}$; interaction egg: modality, $p=0.009$, interaction egg: concentration, $p=0.001$).

Reference LETTUCE 0.1				Reference PE 0.1				Reference PA 0.1				Reference PS 0.1			
	Value	Std.Error	p-value		Value	Std.Error	p-value		Value	Std.Error	p-value		Value	Std.Error	p-value
Intercept	2.9470	0.5764	0.0000	Intercept	4.1079	0.5896	0.0000	Intercept	3.7345	0.5948	0.0000	Intercept	4.4432	0.6108	0.0000
egg number	-0.0137	0.0024	0.0000	egg number	-0.0186	0.0028	0.0000	egg number	-0.0076	0.0024	0.0019	egg number	-0.0157	0.0030	0.0000
Survival PA	0.7874	0.6698	0.2490	Survival L	-1.1609	0.6708	0.0938	Survival L	-0.7874	0.6698	0.2490	Survival L	-1.4962	0.6878	0.0376
Survival PE	1.1609	0.6708	0.0938	Survival PA	-0.3735	0.6871	0.5908	Survival PE	0.3735	0.6871	0.5908	Survival PE	-0.3353	0.7076	0.6390
Survival PS	1.4962	0.6878	0.0376	Survival PS	0.3353	0.7076	0.6390	Survival PS	0.7088	0.7059	0.3233	Survival PA	-0.7088	0.7059	0.3233
Survival 1 %	-0.3094	0.5934	0.6059	Survival 1 %	-0.3094	0.5934	0.6059	Survival 1 %	-0.3094	0.5934	0.6059	Survival 1 %	-0.3094	0.5934	0.6059
Survival 10 %	-0.6806	0.5891	0.2571	Survival 10 %	-0.6806	0.5891	0.2571	Survival 10 %	-0.6806	0.5891	0.2571	Survival 10 %	-0.6806	0.5891	0.2571
egg number: PA	0.0061	0.0030	0.0412	egg number: L	0.0049	0.0029	0.0884	egg number: L	-0.0061	0.0030	0.0412	egg number: L	0.0020	0.0028	0.4869
egg number: PE	-0.0049	0.0029	0.0884	egg number: PA	0.0109	0.0034	0.0014	egg number: PE	-0.0109	0.0034	0.0014	egg number: PE	-0.0029	0.0035	0.4014
egg number: PS	-0.0020	0.0028	0.4869	egg number: PS	0.0029	0.0035	0.4014	egg number: PS	-0.0080	0.0035	0.0220	egg number: PA	0.0080	0.0035	0.0220
egg number: 1 %	0.0068	0.0027	0.0132	egg number: 1 %	0.0068	0.0027	0.0132	egg number: 1 %	0.0068	0.0027	0.0132	egg number: 1 %	0.0068	0.0027	0.0132
egg number: 10 %	0.0089	0.0026	0.0006	egg number: 10 %	0.0089	0.0026	0.0006	egg number: 10 %	0.0089	0.0026	0.0006	egg number: 10 %	0.0089	0.0026	0.0006

Reference LETTUCE 1				Reference PE 1				Reference PA 1				Reference PS 1			
	Value	Std.Error	p-value		Value	Std.Error	p-value		Value	Std.Error	p-value		Value	Std.Error	p-value
Intercept	2.6376	0.5758	0.0000	Intercept	3.7985	0.5889	0.0000	Intercept	3.4251	0.5900	0.0000	Intercept	4.1338	0.6323	0.0000
egg number	-0.0069	0.0022	0.0016	egg number	-0.0118	0.0027	0.0000	egg number	-0.0009	0.0030	0.7753	egg number	-0.0089	0.0030	0.0030
Survival PA	0.7874	0.6698	0.2490	Survival L	-1.1609	0.6708	0.0938	Survival L	-0.7874	0.6698	0.2490	Survival L	-1.4962	0.6878	0.0376
Survival PE	1.1609	0.6708	0.0938	Survival PA	-0.3735	0.6871	0.5908	Survival PE	0.3735	0.6871	0.5908	Survival PE	-0.3353	0.7076	0.6390
Survival PS	1.4962	0.6878	0.0376	Survival PS	0.3353	0.7076	0.6390	Survival PS	0.7088	0.7059	0.3233	Survival PA	-0.7088	0.7059	0.3233
Survival 0.1 %	0.3094	0.5934	0.6059	Survival 0.1 %	0.3094	0.5934	0.6059	Survival 0.1 %	0.3094	0.5934	0.6059	Survival 0.1 %	0.3094	0.5934	0.6059
Survival 10 %	-0.3712	0.5910	0.5347	Survival 10 %	-0.3712	0.5910	0.5347	Survival 10 %	-0.3712	0.5910	0.5347	Survival 10 %	-0.3712	0.5910	0.5347
egg number: PA	0.0061	0.0030	0.0412	egg number: L	0.0049	0.0029	0.0884	egg number: L	-0.0061	0.0030	0.0412	egg number: L	0.0020	0.0028	0.4869
egg number: PE	-0.0049	0.0029	0.0884	egg number: PA	0.0109	0.0034	0.0014	egg number: PE	-0.0109	0.0034	0.0014	egg number: PE	-0.0029	0.0035	0.4014
egg number: PS	-0.0020	0.0028	0.4869	egg number: PS	0.0029	0.0035	0.4014	egg number: PS	-0.0080	0.0035	0.0220	egg number: PA	0.0080	0.0035	0.0220
egg number: 0.1 %	-0.0068	0.0027	0.0132	egg number: 0.1 %	-0.0068	0.0027	0.0132	egg number: 0.1 %	-0.0068	0.0027	0.0132	egg number: 0.1 %	-0.0068	0.0027	0.0132
egg number: 10 %	0.0021	0.0024	0.3791	egg number: 10 %	0.0021	0.0024	0.3791	egg number: 10 %	0.0021	0.0024	0.3791	egg number: 10 %	0.0021	0.0024	0.3791

Reference LETTUCE 10				Reference PE 10				Reference PA 10				Reference PS 10			
	Value	Std.Error	p-value		Value	Std.Error	p-value		Value	Std.Error	p-value		Value	Std.Error	p-value
Intercept	2.2664	0.5665	0.0001	Intercept	3.4273	0.6048	0.0000	Intercept	3.0539	0.5954	0.0000	Intercept	3.7626	0.6031	0.0000
egg number	-0.0048	0.0016	0.0034	egg number	-0.0097	0.0029	0.0009	egg number	0.0013	0.0030	0.6709	egg number	-0.0068	0.0027	0.0128
Survival PA	0.7874	0.6698	0.2490	Survival L	-1.1609	0.6708	0.0938	Survival L	-0.7874	0.6698	0.2490	Survival L	-1.4962	0.6878	0.0376
Survival PE	1.1609	0.6708	0.0938	Survival PA	-0.3735	0.6871	0.5908	Survival PE	0.3735	0.6871	0.5908	Survival PE	-0.3353	0.7076	0.6390
Survival PS	1.4962	0.6878	0.0376	Survival PS	0.3353	0.7076	0.6390	Survival PS	0.7088	0.7059	0.3233	Survival PA	-0.7088	0.7059	0.3233
Survival 1 %	0.3712	0.5910	0.5347	Survival 1 %	0.6806	0.5891	0.2571	Survival 1 %	0.6806	0.5891	0.2571	Survival 1 %	0.6806	0.5891	0.2571

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Survival 0.1 %	0.6806	0.5891	0.2571	Survival 0.1 %	0.3712	0.5910	0.5347	Survival 0.1 %	0.3712	0.5910	0.5347	Survival 0.1 %	0.3712	0.5910	0.5347
egg number: PA	0.0061	0.0030	0.0412	egg number: L	0.0049	0.0029	0.0884	egg number: L	-0.0061	0.0030	0.0412	egg number: L	0.0020	0.0028	0.4869
egg number: PE	-0.0049	0.0029	0.0884	egg number: PA	0.0109	0.0034	0.0014	egg number: PE	-0.0109	0.0034	0.0014	egg number: PE	-0.0029	0.0035	0.4014
egg number: PS	-0.0020	0.0028	0.4869	egg number: PS	0.0029	0.0035	0.4014	egg number: PS	-0.0080	0.0035	0.0220	egg number: PA	0.0080	0.0035	0.0220
egg number: 1 %	-0.0021	0.0024	0.3791	egg number: 0.1 %	-0.0089	0.0026	0.0006	egg number: 0.1 %	-0.0089	0.0026	0.0006	egg number: 0.1 %	-0.0089	0.0026	0.0006
egg number: 0.1 %	-0.0089	0.0026	0.0006	egg number: 1 %	-0.0021	0.0024	0.3791	egg number: 1 %	-0.0021	0.0024	0.3791	egg number: 1 %	-0.0021	0.0024	0.3791

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Annexe 11 (Table A.5.) : Output table of the survival as a response of growth after generalized linear mixed effect modeling. The red lines correspond to the significant endpoints exhibiting differences (significant endpoints: mass, $p=5.62 \cdot 10^{-6}$; interaction mass: modality, $p=0.03$).

Reference LETTUCE 0.1				Reference PE 0.1				Reference PA 0.1				Reference PS 0.1			
	Value	Std.Error	p-value		Value	Std.Error	p-value		Value	Std.Error	p-value		Value	Std.Error	p-value
Intercept	5.09	1.41	0.00	Intercept	8.33	1.62	0.00	Intercept	8.54	1.67	0.00	Intercept	5.46	1.43	0.00
mass	-0.5355	0.2434	0.0286	mass	-0.9732	0.2736	0.0004	mass	-0.9447	0.2832	0.0010	mass	-0.3662	0.2406	0.1291
Survival PA	3.4449	1.6298	0.0430	Survival L	-3.2405	1.7245	0.0700	Survival L	-3.4449	1.6298	0.0430	Survival L	-0.3636	1.2532	0.7737
Survival PE	3.2405	1.7245	0.0700	Survival PA	0.2044	1.9429	0.9169	Survival PE	-0.2044	1.9429	0.9169	Survival PE	2.8769	1.6645	0.0942
Survival PS	0.3636	1.2532	0.7737	Survival PS	-2.8769	1.6645	0.0942	Survival PS	-3.0813	1.5297	0.0530	Survival PA	3.0813	1.5297	0.0530
Survival 1 %	-1.7571	1.4327	0.2296	Survival 1 %	-1.7571	1.4327	0.2296	Survival 1 %	-1.7571	1.4327	0.2296	Survival 1 %	-1.7571	1.4327	0.2296
Survival 10 %	-0.8346	1.4680	0.5739	Survival 10 %	-0.8346	1.4680	0.5739	Survival 10 %	-0.8346	1.4680	0.5739	Survival 10 %	-0.8346	1.4680	0.5739
mass: PA	-0.4091	0.2716	0.1331	mass: L	0.4377	0.2963	0.1407	mass: L	0.4091	0.2716	0.1331	mass: L	-0.1693	0.1944	0.3846
mass: PE	-0.4377	0.2963	0.1407	mass: PA	0.0286	0.3346	0.9320	mass: PE	-0.0286	0.3346	0.9320	mass: PE	-0.6070	0.2818	0.0321
mass: PS	0.1693	0.1944	0.3846	mass: PS	0.6070	0.2818	0.0321	mass: PS	0.5785	0.2500	0.0214	mass: PA	-0.5785	0.2500	0.0214
mass: 1 %	0.2916	0.2428	0.2308	mass: 1 %	0.2916	0.2428	0.2308	mass: 1 %	0.2916	0.2428	0.2308	mass: 1 %	0.2916	0.2428	0.2308
mass: 10 %	0.1100	0.2462	0.6552	mass: 10 %	0.1100	0.2462	0.6552	mass: 10 %	0.1100	0.2462	0.6552	mass: 10 %	0.1100	0.2462	0.6552

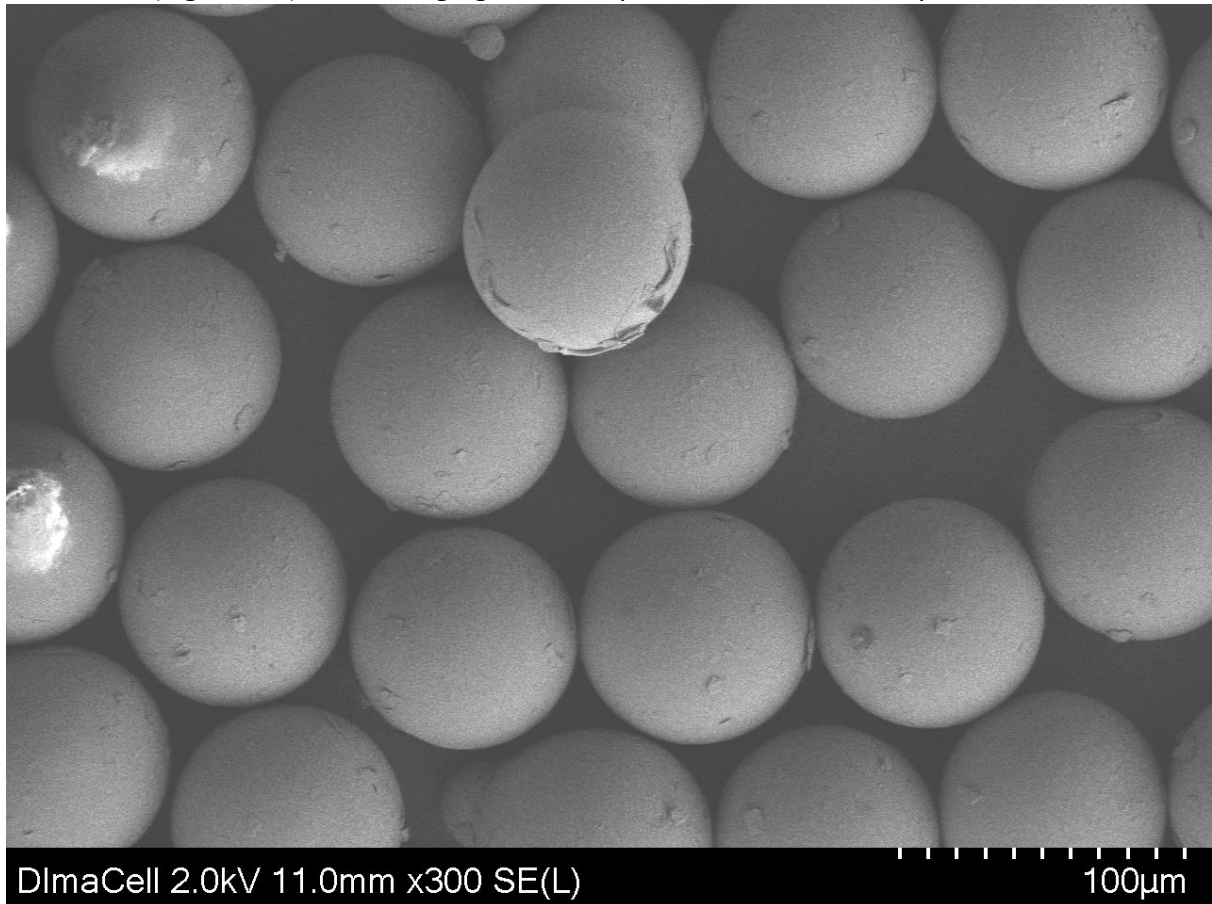
Reference LETTUCE 1				Reference PE 1				Reference PA 1				Reference PS 1			
	Value	Std.Error	p-value		Value	Std.Error	p-value		Value	Std.Error	p-value		Value	Std.Error	p-value
Intercept	3.3360	1.1933	0.0055	Intercept	6.5764	1.5935	0.0000	Intercept	6.7809	1.4195	0.0000	Intercept	3.6996	0.8172	0.0000
mass	-0.2439	0.1929	0.2071	mass	-0.6816	0.2789	0.0151	mass	-0.6531	0.2440	0.0079	mass	-0.0746	0.1022	0.4659
Survival PA	3.4449	1.6298	0.0430	Survival L	-3.2405	1.7245	0.0700	Survival L	-3.4449	1.6298	0.0430	Survival L	-0.3636	1.2532	0.7737
Survival PE	3.2405	1.7245	0.0700	Survival PA	0.2044	1.9429	0.9169	Survival PE	-0.2044	1.9429	0.9169	Survival PE	2.8769	1.6645	0.0942
Survival PS	0.3636	1.2532	0.7737	Survival PS	-2.8769	1.6645	0.0942	Survival PS	-3.0813	1.5297	0.0530	Survival PA	3.0813	1.5297	0.0530
Survival 0.1 %	1.7571	1.4327	0.2296	Survival 0.1 %	1.7571	1.4327	0.2296	Survival 0.1 %	1.7571	1.4327	0.2296	Survival 0.1 %	1.7571	1.4327	0.2296
Survival 10 %	0.9225	1.1186	0.4160	Survival 10 %	0.9225	1.1186	0.4160	Survival 10 %	0.9225	1.1186	0.4160	Survival 10 %	0.9225	1.1186	0.4160
mass: PA	-0.4091	0.2716	0.1331	mass: L	0.4377	0.2963	0.1407	mass: L	0.4091	0.2716	0.1331	mass: L	-0.1693	0.1944	0.3846
mass: PE	-0.4377	0.2963	0.1407	mass: PA	0.0286	0.3346	0.9320	mass: PE	-0.0286	0.3346	0.9320	mass: PE	-0.6070	0.2818	0.0321
mass: PS	0.1693	0.1944	0.3846	mass: PS	0.6070	0.2818	0.0321	mass: PS	0.5785	0.2500	0.0214	mass: PA	-0.5785	0.2500	0.0214
mass: 0.1 %	-0.2916	0.2428	0.2308	mass: 0.1 %	-0.2916	0.2428	0.2308	mass: 0.1 %	-0.2916	0.2428	0.2308	mass: 0.1 %	-0.2916	0.2428	0.2308
mass: 10 %	-0.1815	0.1755	0.3019	mass: 10 %	-0.1815	0.1755	0.3019	mass: 10 %	-0.1815	0.1755	0.3019	mass: 10 %	-0.1815	0.1755	0.3019

Reference LETTUCE 10				Reference PE 10				Reference PA 10				Reference PS 10			
	Value	Std.Error	p-value		Value	Std.Error	p-value		Value	Std.Error	p-value		Value	Std.Error	p-value
Intercept	4.2585	1.0822	0.0001	Intercept	7.4989	1.6220	0.0000	Intercept	7.7034	1.5126	0.0000	Intercept	4.6221	1.0808	0.0000
mass	-0.4255	0.1662	0.0110	mass	-0.8632	0.2815	0.0024	mass	-0.8346	0.2485	0.0009	mass	-0.2562	0.1660	0.1240
Survival PA	3.4449	1.6298	0.0430	Survival L	-3.2405	1.7245	0.0700	Survival L	-3.4449	1.6298	0.0430	Survival L	-0.3636	1.2532	0.7737
Survival PE	3.2405	1.7245	0.0700	Survival PA	0.2044	1.9429	0.9169	Survival PE	-0.2044	1.9429	0.9169	Survival PE	2.8769	1.6645	0.0942
Survival PS	0.3636	1.2532	0.7737	Survival PS	-2.8769	1.6645	0.0942	Survival PS	-3.0813	1.5297	0.0530	Survival PA	3.0813	1.5297	0.0530
Survival 1 %	-0.9225	1.1186	0.4160	Survival 0.1 %	0.8346	1.4680	0.5739	Survival 0.1 %	0.8346	1.4680	0.5739	Survival 0.1 %	0.8346	1.4680	0.5739
Survival 10 %	0.8346	1.4680	0.5739	Survival 1 %	-0.9225	1.1186	0.4160	Survival 1 %	-0.9225	1.1186	0.4160	Survival 1 %	-0.9225	1.1186	0.4160
mass: PA	-0.4091	0.2716	0.1331	mass: L	0.4377	0.2963	0.1407	mass: L	0.4091	0.2716	0.1331	mass: L	-0.1693	0.1944	0.3846
mass: PE	-0.4377	0.2963	0.1407	mass: PA	0.0286	0.3346	0.9320	mass: PE	-0.0286	0.3346	0.9320	mass: PE	-0.6070	0.2818	0.0321
mass: PS	0.1693	0.1944	0.3846	mass: PS	0.6070	0.2818	0.0321	mass: PS	0.5785	0.2500	0.0214	mass: PA	-0.5785	0.2500	0.0214
mass: 1 %	0.1815	0.1755	0.3019	mass: 0.1 %	-0.1100	0.2462	0.6552	mass: 0.1 %	-0.1100	0.2462	0.6552	mass: 0.1 %	-0.1100	0.2462	0.6552
mass: 0.1 %	-0.1100	0.2462	0.6552	mass: 1 %	0.1815	0.1755	0.3019	mass: 1 %	0.1815	0.1755	0.3019	mass: 1 %	0.1815	0.1755	0.3019

Annexe 12 (Table A.6.) : Output table of the mass as a response of the cumulated number of eggs, and of the cumulated number of eggs as a response of mass evolution, after generalized linear mixed effect modeling.

		Chisq	Df	Pr (>Chisq)
mass function of egg number + polymer+ concentration, random effect on the box, quasibinomial distribution)	egg number	2.8732	1	0.09007
	modality	1.8419	3	0.60587
	concentration	1.4127	2	4.93E-01
		Chisq	Df	Pr (>Chisq)
egg number function of mass + polymer+ concentration, random effect on the box, quasibinomial distribution)	mass	3.404	1	0.06504
	modality	2.1319	3	0.54549
	concentration	2.5432	2	2.80E-01

Annexe 13 (Figure S.1) : SEM imaging of the PS particles used in the experiment.



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Annexe 14 (Table S.1) : Metal speciation percentage of each metal used in the spiking solutions. As(V): Arsenate; As(III): Arsenite, AsB: Arsenobetaine, DMA: Dimethyl arsenic acid (or cacodylic acid), Cd(II): Inorganic cadmium, Hg(II): Inorganic mercury, CH₃Hg⁺: Methylmercury

Arsenic	As(V)	AsB	As(III)	DMA
%	92	3	4.5	0.5
Cadmium	Cd(II)			
%	100			
Mercury	Hg(II)		CH ₃ Hg ⁺	
%	98		2	

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Annexe 15 (Table S.2) : Growth rate constants (k_g in d^{-1}) for the different combinations of ME and PS concentrations.

PS As k_g	0			0.01			1		
	0	50	100	0	50	100	0	50	100
	0.044 ± 0.007 ^a	0.067 ± 0.011 ^a	0.070 ± 0.010 ^a	0.068 ± 0.014 ^a	0.048 ± 0.008 ^a	0.053 ± 0.012 ^a	0.053 ± 0.007 ^a	0.047 ± 0.007 ^a	0.051 ± 0.005 ^a
PS Cd k_g	0			0.01			1		
	0	10	20	0	10	20	0	10	20
	0.044 ± 0.007 ^a	0.060 ± 0.011 ^a	0.058 ± 0.009 ^a	0.068 ± 0.014 ^a	0.061 ± 0.010 ^a	0.061 ± 0.012 ^a	0.053 ± 0.007 ^a	0.058 ± 0.008 ^a	0.050 ± 0.007 ^a
PS Hg k_g	0			0.01			1		
	0	2	5	0	2	5	0	2	5
	0.044 ± 0.007 ^a	0.043 ± 0.008 ^a	0.051 ± 0.006 ^a	0.068 ± 0.014 ^a	0.046 ± 0.009 ^a	0.063 ± 0.009 ^a	0.053 ± 0.007 ^a	0.063 ± 0.013 ^a	0.061 ± 0.009 ^a

Annexe 16 : Details on quantification of Eu chelates in PS particles.

The ICP-MS provides results in terms of europium (Eu) concentration per sample. In our study, results are more representative if expressed in mass of NPs per sample. After ensuring that there is no Eu leaching from the Eu-PS particles, Eu concentrations were determined as a function of their mass through a series of dilutions (10^3 , 10^4 and 10^5) of the Eu-PS particles (stock solution) in Hoagland solution and Milli-Q water. This was done by identifying the linear relationship between Eu and Eu-PS particle mass. In summary, we had triplicates for each dilution factor (DF) and triplicates for each type of solution (Hoagland N= 9; and MilliQ N= 9) with known mass-based concentration [1] and we wanted to calculate the Europium concentration [2] for each sample to, hence, build the correlation equation.

A total of 18 samples were divided in three groups depending on their dilution factor that they were subjected to ($\times 10^3$, $\times 10^4$ and $\times 10^5$). Then, each replicate was digested, diluted and treated in the same way as the snail and lettuce samples, and were measured using the ICP-MS (see section 2.3). Then, the correlation equation was built between [1] and [2]. The volume of an ideal particle was then measured ($V_{100} = 5.24 \times 10^{-16} \text{ cm}^3$) and with the Eu-PS NPs density ($\rho_{\text{Eu-PS}} = 1050 \text{ mg/cm}^3$) it was possible to find the mass of particles.

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Annexe 17 (Table S4) : Composition of Hoagland solution (chemicals and micronutrients) and the composition of the different stock solutions used to prepare the ¼ Hoagland solution used in the experiment.

	Chemicals (molar mass)	mg/L
1	Ca(NO ₃) ₂ ·4H ₂ O (236.15)	945
2	KNO ₃ (101.1)	607
3	MgSO ₄ ·7H ₂ O (120.36)	493
4	NH ₄ H ₂ PO ₄ (115.03)	115
	Micronutrients	
5	H ₃ BO ₃ (61.83)	1.48
6	Mn(NO ₃) ₂ ·4H ₂ O (251)	1
7	Zn(NO ₃) ₂ ·6H ₂ O (189.36)	1.19
8	CuSO ₄ ·5H ₂ O (249.68)	0.05
9	MoNa ₂ O ₄ ·2H ₂ O (242)	0.02
10	FeSO ₄ ·7H ₂ O (278)	11.1

Solution A: 94.5 g Ca(NO₃)₂·4H₂O, 60.7g KNO₃ dissolved in 1 L deionized water (100 times)

Solution B: 49.3g MgSO₄·7H₂O, 11.5 g NH₄H₂PO₄ powder dissolved in 1 L deionized water (100 times)

Solution C: 0.1004g Mn(NO₃)₂·4H₂O, 0.119g Zn(NO₃)₂·6H₂O, 0.005g CuSO₄·5H₂O, 0.002g MoNa₂O₄·2H₂O dissolved in 1 L deionized water (400 times)

Solution D: 0.07416g H₂BO₃ dissolved in 500 mL deionized water (800 times), dissolved in hot water.

Solution E: 1.1122g FeSO₄·7 H₂O dissolved in 500 mL deionized water (800 times)

The culture solution (1/4 hoagland solution, pH is measured and adjusted at 6 ± 0.2) is composed by adding 2.5 ml solution A, 2.5 ml solution B, 2.5 ml solution C, 1.25 ml solution D and 1.25 ml solution E to one liter of deionized water.

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Annexe 18 (Figure S1) : Schematic representation of the experimental setup of the direct exposure of lettuce (*Lactuca sativa*) and indirect exposure to a terrestrial snail (*Cornu aspersum*). Lettuce roots were exposed hydroponically to 100 nm europium doped polystyrene (Eu-PS) nanoplastics at different exposure concentrations. After three weeks of exposure, the shoots were then fed to the snails. Samples of lettuce roots and shoots, and the snail's digestive gland and feces were analyzed to measure Eu-PS content.



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Annexe 19 (Figure S2) : The diameter of the snails is measured by the broken line in the image which intersects the shell (Örstan, 2011).



Reference

Örstan, A. (2011). A method to measure snail shell. *Triton*, 23, 1-2.

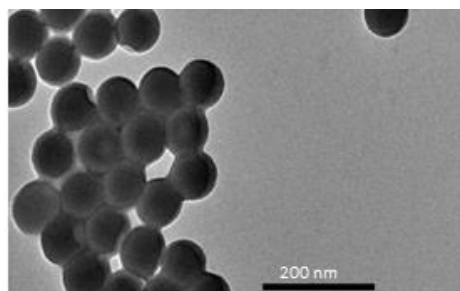
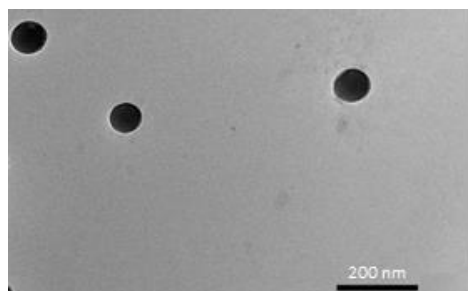
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Annexe 20 (Table S2) : Details about statistical analysis of different endpoints, including if normality and homogeneity were violated, test used, and statistical output.

Species	Endpoint	Assumption violated?	Which one?	Type of test	F-value	p value
Plant	Root dry biomass	No		One way ANOVA	$F_{3,32} = 3.593$	0.0241
	Root wet biomass	No		One way ANOVA	$F_{3,32} = 2.968$	0.0466
	Shoot dry biomass	No		One way ANOVA	$F_{3,26} = 0.552$	0.651
	Shoot wet biomass	No		One way ANOVA	$F_{3,26} = 0.218$	0.883
Snail	Diameter increase	Yes	Normality ($p=0.04$)	Nested ANOVA (effect of beaker)	$F_{3,65} = 1.055$	0.4251
				Nested ANOVA (effect of treatment)	$F_{3,65} = 3.129$	0.0316
	Weight increase	No		Nested ANOVA (effect of beaker)	$F_{3,65} = 1.805$	0.174
				Nested ANOVA (effect of treatment)	$F_{3,65} = 1.396$	0.16
	BSS	Yes	Normality ($p<0.05$)	One way ANOVA (day 7)	$F_{3,20} = 1.118$	0.365
				One way ANOVA (day 14)	$F_{3,20} = 2.053$	0.139
				One way ANOVA (day 19)	$F_{3,20} = 0.566$	0.644
Total leaf consumption	Yes	Normality ($p<0.05$)	One way ANOVA	$F_{3,20} = 1.4$	0.272	
Total feces production	Yes	Normality ($p<0.05$)	One way ANOVA	$F_{3,20} = 1.026$	0.402	

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Annexe 21 (Figure S3) : TEM pictures of our used 100 nm Eu-PS particles: single particles (left photo) and agglomerated particles (right photo).

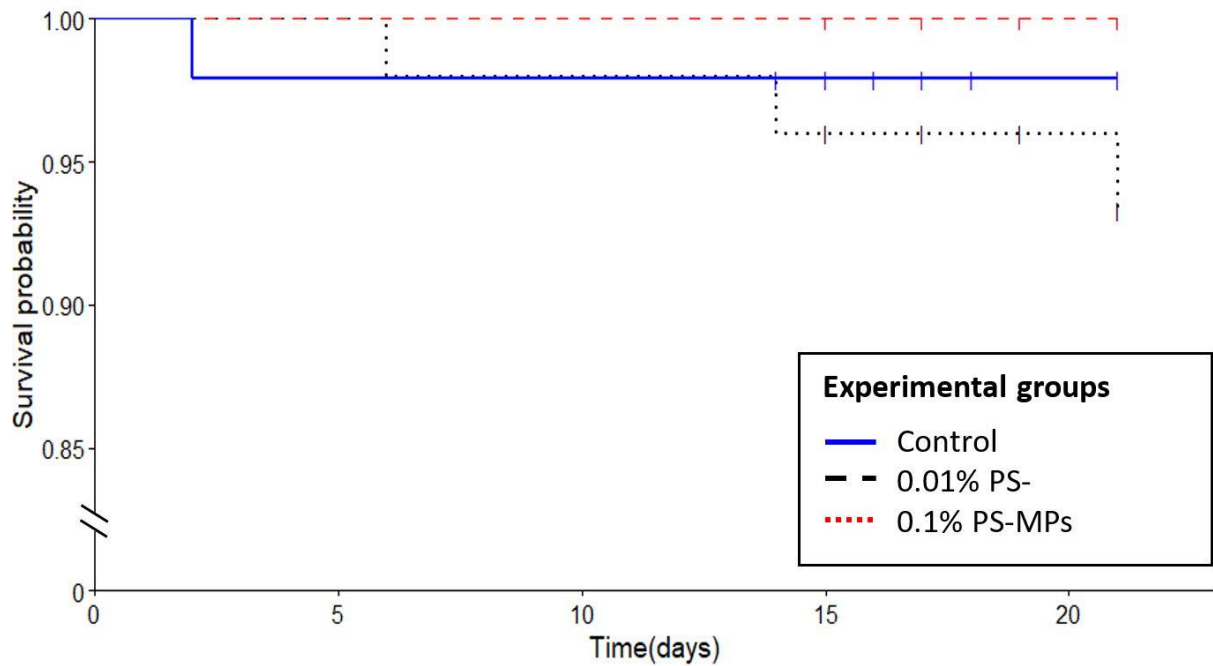


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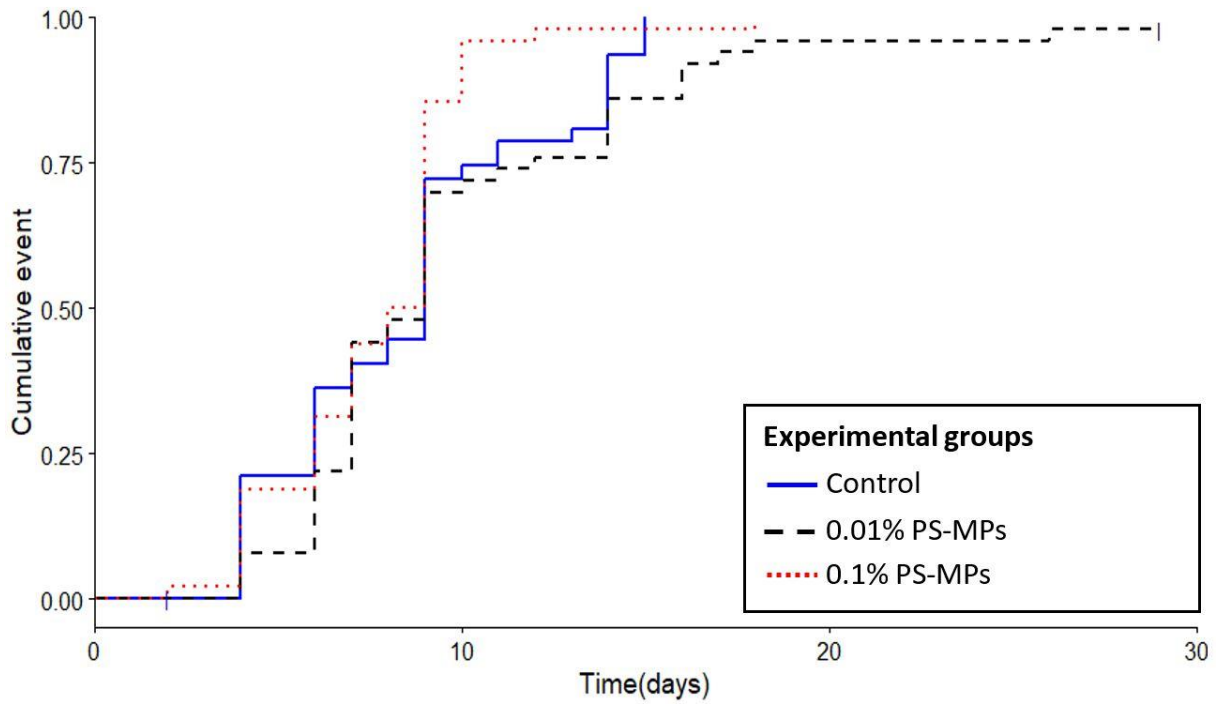
Annexe 22 (Table S3) : Leaching of Eu chelates from the PS nanosphere (0.1% concentration) into Hoagland solution after 1h, 2h, 20h, 24h, 48h and 72h. Samples are represented as mean±SE.

Timepoints	Eu content (ppb)
1 h	0.006±0.0014
2 h	0.012±0.001
20 h	0.031±0.001
24 h	0.031±0.002
48 h	0.037±0.002
72 h	0.049±0.009

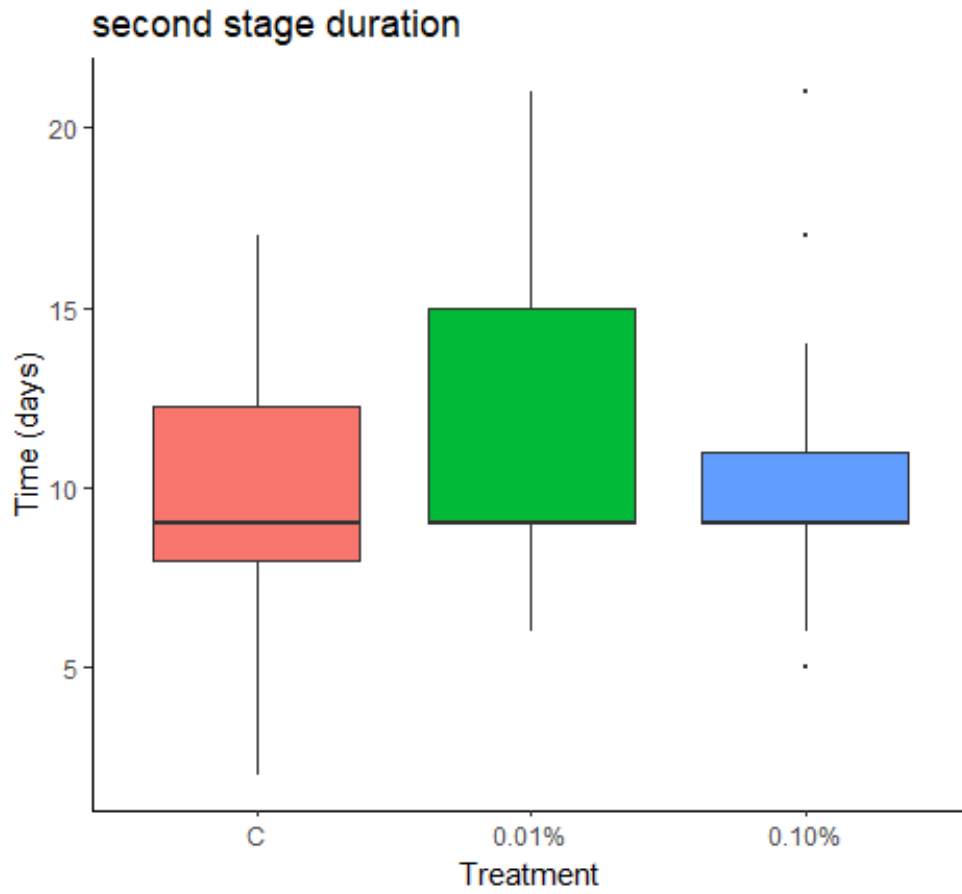
Annexe 23 (Figure S1) : Kaplan-Meier curves illustrating the survival probability of *C. lefebvrei* larvae over 21 days from the individualization (day 0 in the plot), corresponding to the control, 0.01 % and 0.1 % exposed second stage larvae. No significant differences were observed ($p=0>0.05$ for all the comparisons). The curves show breaks (|), when a censoring event occurs.



Annexe 24 (Figure S2) : Kaplan-Meier curves of the *C. lefebvrei* larvae development from hatching (day 0 in the plot) to the first moult, corresponding to the non-exposed first stage larvae. No significant differences were observed ($p=0.9996$). The curves show breaks (|), when a censoring event occurs.



Annexe 25 (Figure S3) : Boxplot of the duration (in days) of the second larval stage of the control, 0.01 % and 0.1 % PS-Eu exposed larvae. No significant differences were observed ($p=0.4691$).





Titre : Evaluation du devenir et de l'impact des microplastiques dans l'environnement terrestre en utilisant l'escargot *Cantareus aspersus*.

Mots clés : Microplastiques, Sols, Toxicité, Transfert, Invertébrés

Résumé : Les écosystèmes terrestres sont considérés comme le plus large réservoir potentiel en microplastiques (MPs). Cependant, il n'existe que peu de données sur la caractérisation de leurs risques dans les sols, et le nombre de modèle biologique utilisé pour leur étude est restreint. L'objectif de ce travail de thèse a été d'étudier le devenir et les effets potentiels des MPs dans l'environnement terrestre en utilisant comme bioindicateur l'escargot *Cantareus aspersus*.

Une première étude a montré des effets écotoxiques des MPs (augmentation ou inhibition de croissance, diminution de la mortalité, induction de stress oxydatif) lors d'exposition sub-chroniques.

Dans une seconde étude, nos données révèlent des atteintes chez l'escargot (augmentation de survie, inhibition de croissance et de reproduction) lors d'expositions à l'échelle du cycle de vie, en lien avec des changements dans l'allocation énergétique des individus entre leurs différentes grandes fonctions biologiques.

Une troisième étude nous a permis d'observer que des concentrations environnementales en MPs entraînent des modifications dans les dynamiques internes en métaux dans l'escargot, tant en termes d'adsorption que d'excrétion, sans changer leur bioaccumulation.

Deux autres études ont révélé et quantifié des transferts trophiques de MPs dans une chaîne alimentaire plante-escargot-prédateur, et ont montré des effets des MPs à tous les niveaux de cette chaîne (diminution de la biomasse végétale, de la croissance de l'escargot, du développement larvaire du prédateur et changement de son statut immunitaire).

En conclusion, nos résultats mettent en lumière la capacité des MPs à être transférés dans les réseaux trophiques et à exercer des effets écotoxiques à tous les niveaux de ces réseaux, en fonction de la taille, la concentration et la nature polymérique des MPs. Ces résultats ouvrent de nouvelles voies vers l'étude des mécanismes sous-jacents impliqués dans ces effets envers le biote.

Title: Assessment of fate and impacts of microplastics in terrestrial environment using the snail *Cantareus aspersus*.

Keywords: Microplastic, Soils, Toxicity, Transfers, Invertebrates

Abstract: Terrestrial ecosystems are the considered the largest potential microplastics (MPs) sink. However, data on their risk characterization are scarcely available, and the number of biological models used in their study is limited. The aim of this thesis work was to identify the fate and potential effects of MPs in terrestrial environments using the *Cantareus aspersus* snail as bioindicator.

A first study showed MPs ecotoxic effects (growth enhancing or inhibition, lessened mortality, oxidative stress induction) during a sub-chronic exposure.

During a second study, the data revealed the snails to be impacted by MPs (enhanced survival, inhibited growth and reproduction) during a life-cycle exposure, linked with trade-offs in the individual energetic allocation between their major biological functions.

A third study allowed to determine that MPs environmental concentrations modified the metal internal dynamics in snails (uptake and excretion) with no change in their bioaccumulation.

Two other studies revealed and quantified MPs trophic transfer in a plant-snail-predator food chain. These studies demonstrated MPs effects at all the levels (vegetal biomass, snail growth and predator development inhibition, and changes in the immunity status of the predator). In conclusion, our results highlights the MPs capacity to be transferred along the food chains and to exert ecotoxic effects at all the levels of these webs, related to the MP size, concentration and polymeric nature. These results pave the way towards the study of the underlying mechanisms governing the MPs effects towards the biota.